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A METHOD FOR ESTIMATING POPULATIONS OF LARVAE OF THE JAPANESE BEETLE IN THE FIELD[1]

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INTRODUCTION

In developing chemical and mechanical methods for controlling the subterranean larvae of the Japanese beetle (*Popillia japonica* Newman) in the field, it is necessary to determine the density of the larval population from time to time to obtain information on the effectiveness of the different treatments. As the third-instar larva of this insect, a white grub about 1 inch long resembling the native white grubs, is easily seen in most soils, it is possible to determine definitely the number of larvae in a given area by loosening all the soil to a depth of 12 inches with a mattock in the fall and spring and counting the larvae. This procedure is not practical, however, when the area is large, because of the enormous amount of labor involved. Moreover, it is not satisfactory in any soil that is to be examined subsequently to determine the effect of an insecticidal treatment, because many of the larvae are killed or injured mechanically during the operation, some migrate to adjacent undisturbed areas, the cover crop, if present, is injured or destroyed, and the effect of the mechanical and chemical treatments in loosened soil is often different from that in soil which has not been disturbed. It was therefore necessary to develop a method of examining small portions of a given area by which larval populations could be estimated without disturbing all the soil.

EXPERIMENTAL PROCEDURE

In October 1934 four plots, each 50 by 50 feet, were selected at Jacobstown, N. J., in a heavily infested locality for a study of methods of sampling. The boundaries of these plots were carefully marked and the change in elevation in inches was determined with a transit theodolite. The vegetation was noted and the position of all prominent plants recorded. Each plot was divided into 1-square-foot sections, and the number of larvae in each square foot was determined by the procedure described above. These data were recorded in their proper positions on charts drawn to a scale of 1 inch to a foot for use in estimating the average populations by different methods.

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DESCRIPTION OF PLOTS

The topography of the experimental plots is shown in figure 1. Plot 1 was in the lowest section of a pasture. The ground was practically level and was uniformly covered with short pasture grasses. The soil was a heavy clay, slightly sandy in spots, and poorly drained. Plot 2, in the same pasture, was sloping and sandy, well drained, and uniformly covered with short pasture grasses. Plots 3 and 4 were practically level cornfields, with a light, well-drained, loamy soil. Corn was planted at intervals of 4 feet over these plots, except in a strip about 5 feet wide on one edge of plot 4, where tomatoes were growing.

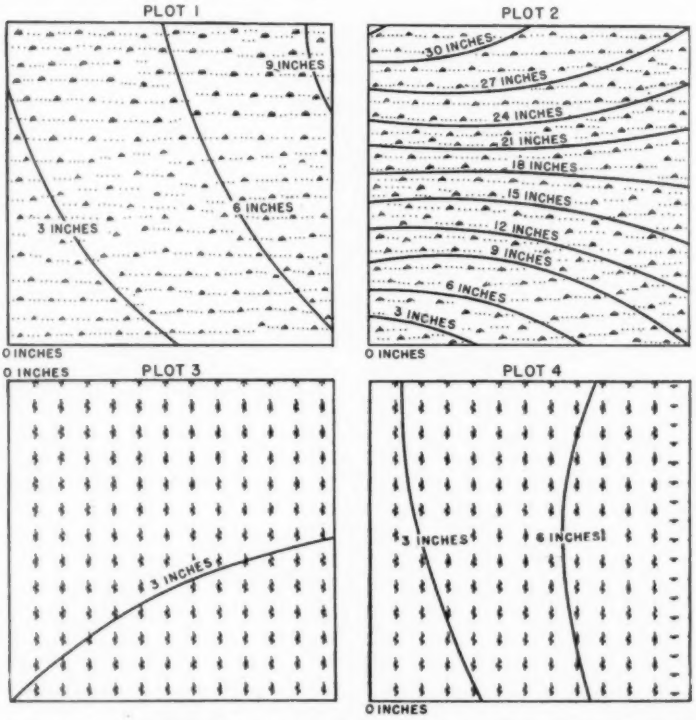

FIGURE 1.—Contour maps of the plots used in estimating populations of Japanese beetle larvae.

LARVAL POPULATION IN THE DIFFERENT PLOTS

The larval populations in the different plots were as follows: Plot 1, 47,866; plot 2, 23,044; plot 3, 12,886; plot 4, 6,909. The larvae were not uniformly distributed over the plots but were more dense in certain sections, as is shown in the dot diagram (fig. 2) for plot 2. The density of the population in the plot ranged from 0 to 46 larvae per square foot. It was not possible to explain this variation by the slight changes in the vegetation or in the elevation of the

land. Such variation of the population in the field is a normal phenomenon.

The numbers of larvae found in each of the 2,500 sections in the different plots were arranged in progressive order and grouped according to the frequency of occurrence. The results are presented graphically in figure 3. It was found that the greater the population in the plot the wider was the variation in the number per square foot and the less frequent was the occurrence of numbers in close proximity to the average. In plot 1, where the population was 47,866 larvae, the number per square foot ranged from 1 to 52,

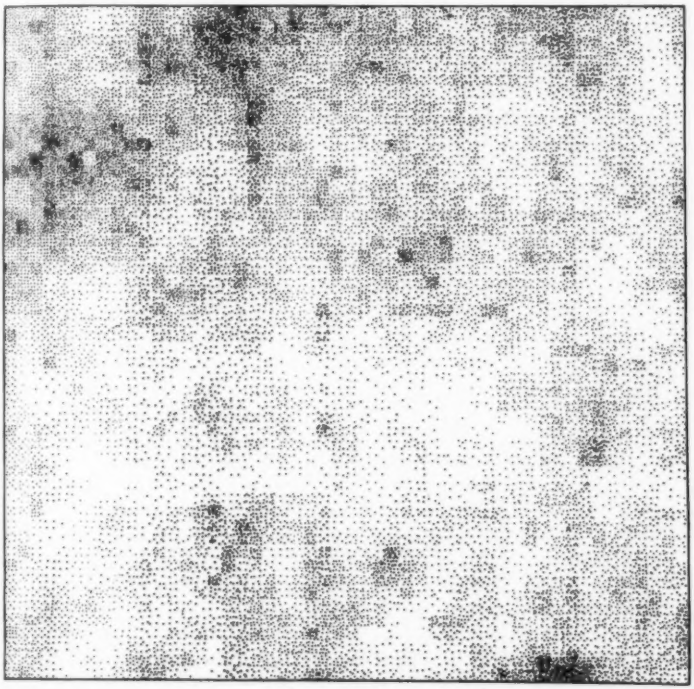

FIGURE 2.—Dot diagram of the distribution of Japanese beetle larvae in plot 2, which was covered with pasture grasses. Each dot indicates one larva.

and 435 cases of the 2,500 were within ± 1 larva of the average, but in plot 4, where the population was 6,909, the variation was from 0 to 20 larvae, and 1,235 cases were within ± 1 larva of the average.

It will be observed (fig. 3) that, with the lightest infestation where many zeros were found, the frequency curve assumed a moderately asymmetrical form. It is to be expected that, when on one side of the greatest frequency the limit is zero and on the other side there is no definite limit, the distribution will be skewed in those

cases where no larvae occur in a large proportion of the units. As the infestation per unit of measurement increased, the zero limit became further removed from the point of greatest frequency, with the result that in the highest infestation the distribution approached the normal symmetrical form. As populations such as those represented in plots 2, 3, and 4 are most frequently encountered in the

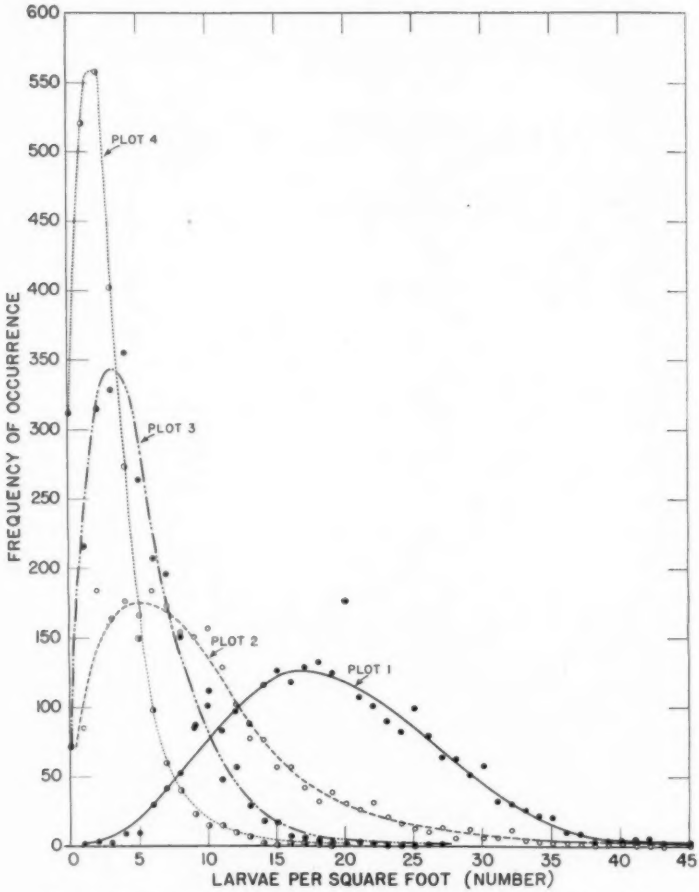

FIGURE 3.—Distribution of larvae of the Japanese beetle in the four plots.

field, it is apparent that the asymmetrical frequency distribution is the most common form in infestations of Japanese beetle larvae.

DETERMINATION OF SIZE OF UNITS TO BE SAMPLED

As the survey charts showed the exact number of larvae in each square foot of each plot, it was possible to combine adjacent 1-square-

foot blocks to form units of any desired size or shape. Units of 1 by 1 foot, 1 by 2 feet, 2 by 2 feet, 1 by 4 feet, 3 by 3 feet, 1 by 9 feet, 1 by 50 feet, and 2 by 50 feet were tried. In the case of the 1- by 4-foot units the width of the rectangles was taken first along the width of the field and then along the length to determine whether the results were significantly changed by modifying the position of these units. The numbers of whole units of these different sizes which could be fitted into a 50- by 50-foot plot were, respectively, 2,500, 1,250, 625, 624, 256, 275, 50, and 25. The average number of larvae and its standard error and the standard deviation, or the error of a single observation, were determined for the different units and are given in table 1. To permit comparison of the errors obtained with the different units, the errors are expressed as relative standard errors, obtained by calculating the standard errors as percentages of the averages.

TABLE 1.—*Statistical constants of larval populations obtained from sampling the entire area of four 2,500-square-foot plots when unit areas of different sizes were used*

Plot no.	Larvae in plot	Size of unit area	Larvae in unit area sampled		
			Mean	Standard deviation	Relative standard error of mean
	Number	*Feet*	*Number*	*Number*	*Percent*
1	47,866	1 by 1	19.15± 0.15	7.44	0.78
		1 by 2	38.30± .38	13.31	.99
		1 by 4 [1]	75.98± 1.00	24.52	1.32
		1 by 4 [2]	76.24± .97	23.81	1.27
		2 by 2	76.59± .97	24.26	1.27
		1 by 9	167.01± 3.24	51.24	1.94
		3 by 3	169.34± 3.34	53.28	1.97
		1 by 50	957.40±33.20	232.43	3.47
		2 by 50	1,914.16±93.60	458.54	4.89
2	23,044	1 by 1	9.22± .14	6.87	1.52
		1 by 2	18.43± .36	12.78	1.95
		1 by 4 [1]	36.68± .97	23.65	2.64
		1 by 4 [2]	36.15± .95	23.22	2.63
		2 by 2	36.87± .97	24.20	2.63
		1 by 9	78.57± 2.59	40.91	3.30
		3 by 3	85.63± 3.02	48.17	3.53
		1 by 50	460.68±13.40	93.81	2.91
		2 by 50	921.56±41.77	204.64	4.53
3	12,886	1 by 1	5.15± .07	3.53	1.36
		1 by 2	10.31± .18	6.20	1.75
		1 by 4 [1]	20.30± .43	10.50	2.12
		1 by 4 [2]	20.07± .43	10.50	2.14
		2 by 2	20.62± .42	10.50	2.04
		1 by 9	45.37± 1.29	20.44	2.84
		3 by 3	44.54± 1.34	21.35	3.01
		1 by 50	257.72± 9.71	67.98	3.77
		2 by 50	515.44±26.73	130.96	5.19
4	6,909	1 by 1	2.76± .05	2.43	1.81
		1 by 2	5.52± .12	4.16	2.17
		1 by 4 [1]	11.25± .29	7.05	2.58
		1 by 4 [2]	10.91± .31	7.58	2.84
		2 by 2	11.05± .30	7.61	2.71
		1 by 9	24.03± .92	14.47	3.83
		3 by 3	25.04± .92	14.70	3.67
		1 by 50	138.38± 3.92	27.44	2.83
		2 by 50	276.36±11.16	54.68	4.04

[1] Width taken along width of field.
[2] Width taken along length of field.

DETERMINED FROM STANDARD DEVIATION FROM THE MEAN

The standard deviation (σ) is the constant usually employed to measure the degree of dispersion of the variates about the average, or mean, of the group. Theoretically about 68 percent of the values should lie within a range of 1 standard deviation above and below the mean. To determine the reliability of this measure of dispersion for the different units used in this study, the percentage of the samples in each unit which was within this range was calculated for the plot of heaviest infestation. It was found that 69 percent of the 1- by 1-foot units were within $\pm$ 1 standard deviation of the average. The results with the other units were as follows: 1 by 2 feet, 67 percent; 2 by 2 feet, 64 percent; 3 by 3 feet, 62 percent; 1 by 50 feet, 58 percent; 2 by 50 feet, 56 percent. It is apparent that the standard deviation is an accurate measure of the degree of dispersion when a large number of samples are involved, as in the 1- by 1-foot units, but that it becomes less accurate as the size of the unit is increased, and the number required to cover a given area is decreased. With the larger units the standard deviation indicated a smaller dispersion than actually occurred.

DETERMINED FROM THE STANDARD ERROR OF THE MEAN

When the relative standard errors of the means of the different units are compared, it is apparent that the 1- by 1-foot unit is the most accurate for estimating the number of larvae. As the size of the unit is increased, the relative standard error of the mean becomes larger. With the 1- by 50-foot and the 2- by 50-foot units the relative standard errors do not appear to be correlated with the density of the larval population. The relative standard errors of the small units become greater with the decrease in the number of larvae per unit. It is apparent that when many larvae are present the average population per unit of measurement can be estimated more accurately than when only a few larvae are in the plot.

DETERMINED FROM CONCENTRATION AROUND MEDIAN VALUE

Before this study was undertaken, it was the general opinion that larval populations could be estimated more accurately with square-yard units than with square-foot units. It can be demonstrated with the heaviest infestation, in plot 1, that square-foot units give more accurate measurements because they are grouped more closely about the median value than are the larger units. When the number of larvae per unit is plotted against the frequency of occurrence for units of 1 by 1, 1 by 2, 2 by 2, and 3 by 3 feet, as in figure 4, it will be observed that the values for the 1- by 1-foot units are closely grouped about the median value. As the size of the unit is increased the centralizing tendency gradually disappears, until with the 3- by 3-foot units the values are scattered almost uniformly along the base line. There is no centralizing tendency with the 1- by 50-foot and the 2- by 50-foot units. It is obvious that an average can be estimated most accurately with units that arrange themselves close to the median.

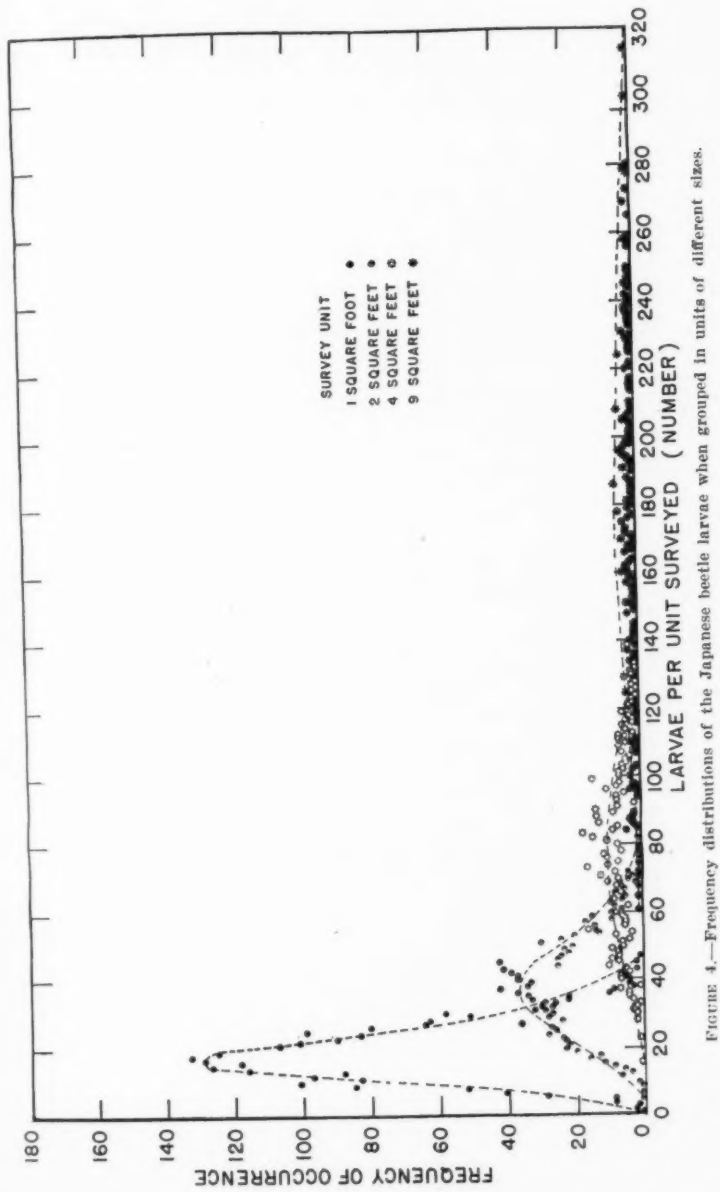

FIGURE 4.—Frequency distributions of the Japanese beetle larvae when grouped in units of different sizes.

EFFECT OF SHAPE OF UNITS

The data in table 1 show that the 1- by 4-foot units gave approximately the same results as the 2- by 2-foot units and that the results with the 1- by 9-foot units were about the same as those with the 3- by 3-foot units. It may be concluded, therefore, that the size, and not the shape, of the units is the determining factor.

CONCLUSION AS TO BEST SIZE OF UNIT

It may be concluded, from a consideration of the standard deviations, the standard errors of the means, and the concentration of the units about the median, that the 1- by 1-foot unit, which is the smallest practical unit for digging in the field, is the most dependable for estimating larval populations in the field.

DETERMINATION OF PERCENTAGE OF AREA TO BE SAMPLED

DETERMINED FROM STANDARD ERRORS OF THE MEANS

If the standard deviation [2] as determined for the whole plot with any of the above-mentioned units is assumed to be a constant for that unit in the plot, it is possible to estimate the standard errors of samples covering definite percentages of the plot by substituting the different values for n in the equation $\sigma=\frac{\sigma_x}{\sqrt{n-1}}$

For example, if an entire plot was considered in 1- by 1-foot units, n would be 2,500, but if 1 percent of the plot was used, n would be 25. If the plot was taken in 3- by 3-foot units, n would be 256. The standard errors of the means were determined when different percentages of the plots were considered to be covered uniformly with units of the different sizes, and expressed as "relative standard errors" to permit comparison of the errors obtained with the different units. These data are shown graphically in figure 5.

It will be noted from figure 5 that with each unit the relative standard error increases rapidly when the percentage of the area sampled becomes small. The beginning of this rapid increase depends not only on the size of the unit but on the density of the larval population. If it is desired to estimate the average population of a unit area from any of the four plots within a relative standard error of approximately 20 percent, it will be necessary to take about 0.9 percent of the area in 1- by 1-foot units (*a*), 1 percent in 1- by 2-foot units (*b*), 2 percent in 2- by 2-foot units (*c*), 4 percent in 3- by 3-foot units (*d*), 6 percent in 1- by 50-foot units (*e*), and 10 percent in 2- by 50-foot units (*f*). In other words, if 10 percent of the area used in the 2- by 50-foot units was divided into 1- by 1-foot units and distributed at random over the plots, the relative standard error would be reduced from almost 20 percent to less than 6 percent.

EFFECT OF LARVAL POPULATION

As previously stated, the relative standard error is affected by the density of the larval population. When the plots were divided into 1- by 1-foot units, the relative standard error for plot 1 was

[2] Computed by the formula $\sigma_x=\sqrt{\frac{\Sigma x^2}{n}}$.

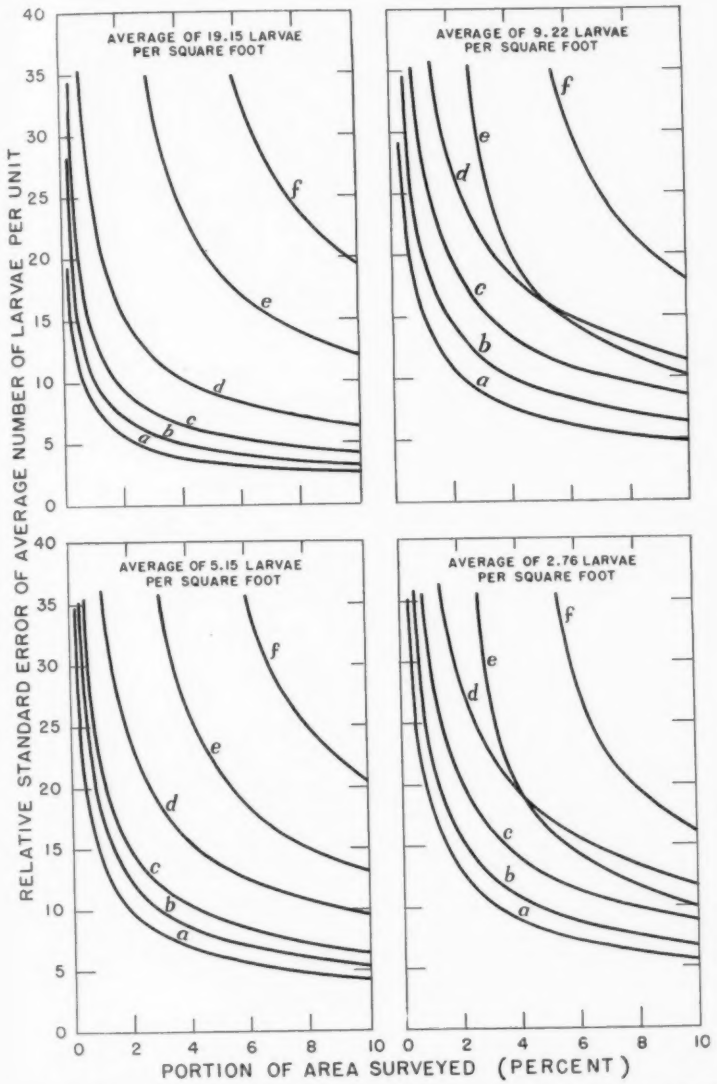

FIGURE 5.—Relative standard error of the mean number of Japanese beetle larvae in the soil when different units were used in making the survey and different percentages of the plot were covered by the sample: *a*, 1 by 1 foot; *b*, 1 by 2 feet; *c*, 2 by 2 feet; *d*, 3 by 3 feet; *e*, 1 by 50 feet; *f*, 2 by 50 feet.

0.78 percent with an average of 19.15 larvae and for plot 4, 1.81 percent with an average of 2.76 larvae (table 1); when 1 percent of these plots was taken in these units, the relative standard errors were, respectively, 7.92 and 17.98 percent (fig. 5). There seemed to be a straight-line relationship between the relative standard error and the larval population. The lines showing the relationship for the 1- by 1-foot units when different percentages of all the plots were surveyed were determined by the method of least squares, and the results are shown graphically in figure 6. It may be predicted, from the projections of the lines to the right beyond the points of actual determination (shown in broken lines in the figure), that only a small number of 1- by 1-foot units would be required to estimate dense populations ranging from 30 to 40 larvae per square foot. However, since the average number of larvae encountered in most control investigations ranges from 1 to 10 per square foot, and since it is desirable to estimate the average within a possible error of 20 percent, it was decided that at least 1 percent of the area in 1- by 1-foot units should be used in estimating populations of the larvae of the Japanese beetle.

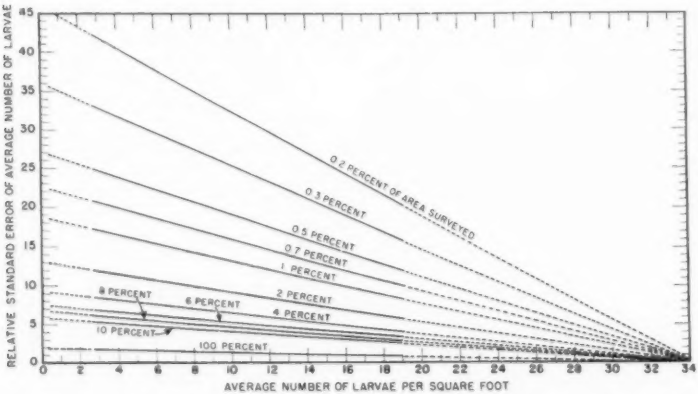
RELATIVE STANDARD ERROR OF AVERAGE NUMBER OF LARVAE
AVERAGE NUMBER OF LARVAE PER SQUARE FOOT
0.2 PERCENT OF AREA SURVEYED
0.3 PERCENT
0.5 PERCENT
0.7 PERCENT
1 PERCENT
2 PERCENT
4 PERCENT
6 PERCENT
8 PERCENT
10 PERCENT
100 PERCENT
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
0 5 10 15 20 25 30 35 40 45

FIGURE 6.—Relation between the relative standard error of the mean number of larvae and the larval population when different percentages of the area were sampled in 1- by 1-foot units.

When the size of the plot is less than 2,500 square feet, it is suggested that a minimum of 25 units be used in the sample. The method of obtaining samples from large areas will be governed to a large extent by the nature of the problem, the local conditions, and the facilities for conducting the work. It is suggested that, when the larval population is to be estimated in an area covering several acres, plots covering approximately 2,500 square feet each be laid out, with at least one plot in a representative part of each acre. After the larval population in each of these representative plots has been estimated accurately, it is possible to estimate with a minimum of labor the population of the whole area and to determine the significance of the difference between different sections.

RELIABILITY OF ESTIMATING LARVAL POPULATIONS FROM 1-SQUARE-FOOT DIGGINGS

In any sampling procedure it is assumed that there is a large "universe" of uniform conditions, in that the individual units vary among themselves in response to the same causes and with about the same variability. The units in a sample should be so selected that the chances of a high value being followed by another high value will be the same as of a low or median value being followed by a high value. When diggings are being made to estimate the number of larvae of the Japanese beetle, there is a tendency to leave those sections where few larvae are found and to continue in those sections where several larvae are found in each digging. When the units of a sample are obtained in this manner, the sample cannot be considered as having been taken at random and the results will not be an accurate estimate of the true average. To eliminate the possibility of biased sampling, it was decided to take the units of a sample at definite intervals over the whole area. When 1 percent of a plot containing 2,500 or more square feet was sampled, the 1-square-foot diggings were made at intervals of 10 feet.

The reliability of this method of sampling was tested by determining the number of samples taken from the four experimental plots which lay outside a specified range from the true average. The average populations of larvae in these plots, as determined from the whole areas, were 19.15, 9.22, 5.15, and 2.76 per square foot (table 1). As the variability of the mean is inversely proportional to the square root of the number of units in the sample, the errors derived from 1 percent of the plots are larger than those given in table 1, which were derived from the whole plot. The standard errors derived from 1 percent of the plots were 1.51, 1.40, 0.71, and 0.49, respectively. One hundred samples, each consisting of twenty-five 1- by 1-foot units, were taken at 10-foot intervals in each plot and the average of each sample was determined. The percentage of cases in which the estimated average was more than 0.5, 1, 1.5, 2, etc., times the standard error of the true average is given in table 2. When the true average was 19.15, 38 percent of the estimated averages differed from the true average by more than 0.5 times the standard error, and in only 1 percent was the variation more than two times the standard error. The variations in the extent to which the estimated values varied from the true averages in the different plots are probably not significant.

The general probability that the estimated average departs from the true value of the universe by more than the stated multiple of the standard error was determined by averaging the departures noted for the different plots. These data are given in the last column of table 2 and presented graphically in figure 7.

It was found that with 1 standard error an average reliability of 75.5 percent was obtained; that is, there were 24.5 chances out of 100 that the true average would not come within the range covered by the estimated average $\pm$ 1 standard error. The reliability was 96.5 percent when the standard error was multiplied by 2, thus making the chances rather remote that the true average would not come within the range covered by two times the standard error.

TABLE 2.—*Probability that an average computed from 1-square-foot diggings, taken at intervals of 10 feet with a minimum of 25 diggings, lies farther from the true average than the stated times the computed standard error, for different larval populations in the soil*

Number of times standard error	Probability of estimated average lying farther from true average than stated number of times standard error when true average was—				
	19.15±1.51	9.22±1.40	5.15±0.71	2.76±0.49	Average
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
0	100	100	100	100	100.00
0.5	38	54	46	61	49.75
1	11	24	24	39	24.50
1.5	3	10	7	16	9.00
2	1	5	3	5	3.50
2.5	0	0	2	1	.75
3	0	0	1	0	.25
3.5	0	0	1	0	.25
4	0	0	0	0	.00

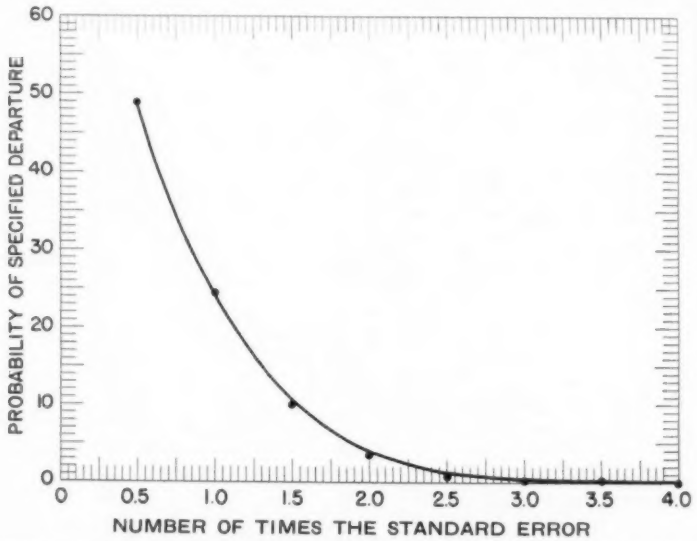

FIGURE 7.—General probability that an average computed from a sample lies farther from the true value than the stated number of times the standard error for populations averaging between 2.76 and 19.15 larvae per square foot when a minimum of 25 units constitutes the sample.

When it is desired to estimate a larval population with a high degree of accuracy, it is suggested that the area be sampled sufficiently to reduce the relative standard error to less than 10 percent. Thus, if 1 percent of a plot is sampled and the average population is estimated as 10 larvae per square foot, it will be seen from figure 6 that the average relative error for this density of population and proportion of the area would be approximately 14 percent, indicating that the sampling should be more extensive. In this case it would be desirable to continue the sampling until 2 percent of the plot had

been examined. If the sample in the example covered 2 percent of the area, and if the standard error of the final estimate was multiplied by 2, it would be expected that the estimated average would have a reliability of 96.5 percent and that twice the relative standard error would be less than 20 percent.

SUMMARY AND CONCLUSIONS

A method has been developed for estimating the population of larvae of the Japanese beetle in the field. The number of larvae in each square foot of four 2,500-square-foot plots was determined, and the results were used in estimating the true averages in each plot by different methods.

The 1-square-foot unit was found to be the most accurate for estimating the population. As the size of the sampling unit was increased, the error became progressively larger. The size, and not the shape, of the sampling unit was the modifying factor.

The error of the estimate is influenced to some extent by the density of the population. It is possible to estimate a dense population more accurately than a sparse population.

It is recommended that in estimating the larval population a minimum of 25 units of 1 square foot uniformly distributed constitute a sample from plots containing less than 2,500 square feet, and in larger areas that the units be spaced not more than 10 feet apart. The larval populations can be estimated most accurately with a minimum of labor in large areas covering several acres by estimating the number of larvae in 2,500 1-square-foot plots placed in representative portions.

By proper sampling it was found to be possible to obtain a reliable estimate of the larval population in a given area.

ALFALFA DWARF, A VIRUS DISEASE TRANSMISSIBLE BY GRAFTING[1]

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INTRODUCTION

Alfalfa dwarf was reported and described for the first time by the writer (*11*)[2] in 1931 as a disease of unknown etiology. In a later paper (*12*) he reported that temperature, soil moisture, soil fertility, and the cutting of plants in an immature stage were not causal factors, nor was the disease due to an excess or deficiency of boron, manganese, strontium, bromine, titanium, iodine, aluminum, copper, or zinc. These conclusions are confirmed by the following field observations: (1) Dwarf does not attack plants uniformly in certain areas; (2) it is most destructive where growth is best; and (3) soils on which the disease is most destructive grow luxuriant alfalfa at each reseeding.

Concomitant with the studies which showed that dwarf is not due to these environmental and cultural factors, experiments were conducted to determine whether the disease is due to some micro-organism or to a virus. Since this project has now been discontinued, the results of these experiments are reported in this paper.

METHODS

The alfalfa (*Medicago sativa* L.) plants used in these experiments, unless otherwise stated, were of the Arizona Common or Hairy Peruvian varieties, and they were grown in pots, or in small basins on land that had never grown alfalfa, or in the field. In most cases they were less than a year old and, as nearly as could be determined, free of dwarf or other root diseases. After the plants were inoculated with bacteria, by grafting or otherwise, as hereinafter described, they were replanted and allowed to grow for several months before they were examined. In some of the experiments plants grown in the field were inoculated without being removed from the soil.

Unless otherwise stated, the work with potted plants was done in the open on the campus of the University of California, Riverside, Calif., nearly a mile from the nearest alfalfa field, and pots of different sizes as well as 16-gallon garbage cans were used.

When uninfested soil was desired, 4 to 6 inches of surface soil was removed from an area on the mountainside, where no cultivated crop had ever grown, and the subsoil thus exposed was used. The fact that no infection from this subsoil was ever observed in the controls attested to the safety of this procedure. Soil was taken from about diseased plants to determine the presence or absence of the causal agent in it.

[1] Received for publication Feb. 4, 1936; issued October 1936. Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Division of Agronomy, California Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 346.

Many plants were set in basins made on land that had never grown alfalfa. These basins were usually 4 by 6 feet and were constructed by removing about 6 inches of the surface soil and placing it about the margin of the area in order to retain the irrigation water and thus permit independent watering of the basins. No evidence of the disease spreading from one basin to another was ever observed.

ISOLATIONS AND INOCULATIONS

Isolations were made from nearly 1,000 dwarfed alfalfa plants, having all stages of disease, and from all parts of the plants and at every season of the year. Both naturally infected plants and those having the disease as a result of grafting were studied. A large number of media were tried, including several liquid media as well as many of the commonly used agars, with different amounts and sources of carbon and nitrogen and various hydrogen-ion concentrations. The isolations were made in different ways, including poured plates and tissue plantings. Anaerobic conditions were supplied in some instances. In a few cases a sterile capillary glass tube was stirred about in a plugged duct under aseptic conditions and the tube was wiped over the surface of an agar plate. Some of the contents of the plugged ducts were drawn into the tube and discharged on the surface of agar in plates. The manipulation of the glass tube was accomplished by a slight modification of the method described by Roberts (*10*) for picking up single spores. Sterile distilled water was drawn through segments of diseased roots by means of suction under aseptic conditions, and the water was plated on agar. Numerous other measures were tried in an attempt to isolate a causal micro-organism from the diseased tissue, but it is not necessary to discuss these in detail. Suffice it to say that these isolations failed to yield with any degree of consistency any one organism, or one that was capable of causing infection under the experimental conditions provided. Bacteria did appear in about 75 percent of the plates, but similar-appearing organisms were often obtained from control isolations from healthy tissue. In by far the larger number of cases the isolations were made from the wood of the roots, as this was the tissue most conspicuously affected. A high percentage of the plantings from the wood of the root after the bark was peeled back aseptically gave bacteria. Poured plates commonly remained sterile or gave only one or a few scattered colonies, often no two being alike. A single planting from a diseased root often gave two or more species of bacteria, indicating that the alfalfa roots affected with dwarf soon were invaded with secondary bacteria. Fungi also were sometimes present.

Inoculations were made only with the bacteria appearing most consistently in the isolations, some of them being used several times. In a number of instances several or all of the bacteria obtained on a set of plates were washed into a beaker with sterile distilled water and used as inoculum. Plants that were wounded, not only by breaking the roots when they were removed from the soil but also by cutting the taproot beneath the suspension, were stood in the suspension for 2 hours or longer and then replanted. In some cases the plants were inoculated in place by (1) pricking a broth culture into the taproots or stems, (2) cutting off the stems with a razor and, as quickly as

possible, placing some of the broth culture on the fresh wounds with a pipette dropper, or (3) dipping pieces of healthy wood into bacteria on plates or slants and inserting them into wounds in taproots of healthy plants. Small pieces of diseased roots, cut as nearly aseptically as possible, were dropped into flasks of nutrient broth or sterile distilled water, and after 24 hours or longer healthy plants were stood in the culture for a time and then planted. In other instances pieces of cotton were first dipped into the broth culture and then forced into the taproot of a healthy plant and allowed to remain. Not a single plant inoculated with bacteria became diseased. The results of the isolations and inoculations seem to justify the conclusion that alfalfa dwarf is not caused by a micro-organism that can be handled by the methods generally used in bacterial plant pathology. The conclusion that dwarf is not caused by a bacterium is strengthened by experiments dealing with the method by which the disease is spread, which will be discussed later in this paper.

TRANSMISSION EXPERIMENTS WITH VARIOUS AGENCIES

SOIL

The first experiments were planned to determine whether dwarf is soil-borne. Soil from about the roots of badly diseased plants from different sources was placed in pots, in 16-gallon cans, or in basins constructed as described under Methods. Healthy plants or seeds were planted in this soil and allowed to grow in the open for various periods. Controls consisting of healthy plants or seeds planted in mountain soil were run in most cases. Sometimes part of the soil was sterilized with formaldehyde (250 cc of 40-percent formaldehyde in 1 gallon of water) before planting, the unsterilized part being used as a control. The data obtained are given in table 1.

TABLE 1.—*Effect of setting healthy alfalfa plants in soil from about diseased plants*

Date plants set out	Date final notes taken	Source of soil	Soil treatment	Plants showing indicated condition at end of experiment	
				Healthy	Diseased
				Number	*Number*
Apr. 4, 1929	Oct. 18, 1930	Chino	Not sterilized	4	0
		do	do	24	0
Oct. 30, 1929	July 10, 1933	Riverside	do	33	0
		do	Sterilized	8	0
		Arlington	Not sterilized	25	0
		do	Sterilized	50	0
Oct. 30, 1930	do	Riverside	Not sterilized	9	0
Sept. 8, 1931	do	do	do	22	0
Sept. 12, 1932	Aug. 6, 1934	do	do	18	4
		do	do	22	1
		Original mountain soil in basin used as control for other 2 experiments of same date.	do	22	1

Only in the experiment set up on September 12, 1932, did any infection develop in the soil in which dwarfed plants had grown. In this experiment the soil in two basins, each about 4 feet square, was removed and replaced to a depth of 1 foot with soil from about badly diseased plants from two different fields. The original soil was left

in a third basin as a control. Twenty-five young healthy plants were set in each basin. At the end of the first year no disease was apparent in any of the basins, but after 2 years 4 out of 22 plants and 1 out of 23 plants were diseased in the two basins in which the soil had been replaced, while 1 diseased plant appeared in the control basin. Since one plant developed the disease through natural infection in the control basin, it is possible, and even probable, that the five infected plants in the other two basins also were infected by natural means and not by soil from about diseased plants.

In addition to these experiments, some evidence that dwarf is not soil-borne was obtained in a study of insect transmission. Approximately 200 healthy plants set under cages in soil that had grown badly dwarfed plants for the 2 preceding years were still healthy at the end of July of the second growing season.

The data given above seem to show fairly conclusively that the causal agent of dwarf does not live, at least to any considerable extent, in soil that is free of affected plant tissue. It also lends strength to the view that dwarf is not caused by an unsuitable soil condition, such as an excess or a lack of certain necessary or toxic chemicals.

DISEASED PLANTS

Since dwarf did not appear to be transmitted through the soil, experiments were designed to determine whether the disease would spread from a diseased to a healthy plant. The results of these experiments are recorded in table 2.

TABLE 2.—*Spread of dwarf from diseased to healthy plants*

Date plants set out	Date final notes taken	Plants inoculated	Control plants	Plants infected			
				Inoculated		Control	
		Number	*Number*	*Number*	*Percent*	*Number*	*Percent*
Dec. 2, 1930	June 9, 1932	10	10	8	80	0	0.0
Feb. 27, 1930	July 3, 1931	20		16	80		
Feb. 17, 1931	Sept. 22, 1931	50	250	13	26	0	.0
Mar. 1, 1934	Sept. 21, 1934	200	200	30	15	1	.5
Sept. 30, 1932	Aug. 20, 1934	48	75	11	23	0	.0
May 16, 1934	May 28, 1935	46	60	16	35	0	.0
		7	[1] 7	2	29	0	.0

[1] Entire diseased plants were killed by boiling before being planted.

In the first experiment four 16-gallon cans filled with mountain soil in which badly diseased plants had been growing for the 2 preceding years were used. The dwarfed plants still alive were removed from two of the cans, while those in the other two cans were allowed to remain. Five healthy plants were set in each can on December 2, 1930, and by June 9, 1932, four out of five, or 80 percent, of the new plants in each of the two cans from which the dwarfed plants had not been removed were affected with dwarf, whereas no disease had developed in the other two cans. These two sets of cans differed only in the fact that the diseased plants were present in one set. The presence of these diseased plants resulted in the difference between 80-percent infection and none at all, thus suggesting that the causal agent survives only in living tissue.

Further evidence of the rapidity of the spread of dwarf from diseased to healthy plants was obtained by setting diseased and healthy plants alternately in pots, cans, basins, or rows in the field. Comparable controls of healthy plants only were run. In the field or in the basin the plants were set 6 inches apart in the row. As shown in table 2, the percentage of infection obtained varied from 15 to 80 percent. In only one case did a control plant become infected.

Although designed for another purpose, the results of an experiment set up on September 30, 1932, give additional data on the subject under discussion. Forty-eight healthy plants were set on one side of a 3-foot board partition and 52 diseased plants on the other side. The partition kept the tops from intermingling but not the roots. On August 20, 1934, 37 of the 48 originally healthy plants remained healthy and 11, or 23 percent, were diseased. Seventy-five healthy plants growing in a basin nearby as controls remained healthy.

These experiments show that dwarf does spread from plant to plant, but they do not reveal the method by which the causal agent is transferred from diseased to healthy plants.

WATER

Previous experiments (*12*) have shown that the severity of alfalfa dwarf is greatly influenced by soil moisture, the disease being more destructive on soil kept sufficiently wet for vigorous plant growth than on dry soil. Frequently the disease appears first in severe form about standpipes or where water is applied most heavily. This suggests the possibility that the disease is distributed by water.

One experiment designed to throw light on this point and initiated February 17, 1931, consisted in the use of three basins situated at different levels on a mountainside where cultivated crops had never been grown. One hundred young healthy plants each were set in the upper and lower basins, and 50 diseased plants in the center basin. The upper basin was watered independently and served as a control. The lower basin was watered by the overflow from the center basin. No dwarf had developed in either of the end basins when the experiment was discontinued on October 6, 1933.

Alfalfa was grown in two rows, each about 150 feet long, on land where it had not been grown before. Diseased plants from the field were set in the 50 feet of row 1 nearest the irrigation ditch, and seed was sown in the remainder of the row. Row 2 was divided into sections that were planted with seed and diseased plants alternately, so that there were four sections sown with seed and three set with diseased plants. The plants were set in the bottom of furrows 6 inches deep so that the irrigation water would come into contact with the crowns of the plants. The plants grown from seed in the first 15 feet of row 2 nearest the irrigation ditch received water that had not come in contact with the diseased plants; however, the second, third, and fourth lots of healthy plants (grown from seed) in this row received water that had first passed around one, two, and three sections of 25 diseased plants, respectively. At the end of 1 year half as many plants affected with dwarf appeared at the end of row 2 next to the irrigation ditch as at the other end, and the disease had spread to three rows of alfalfa in an adjoining plot that had received no water previously in contact with diseased plants. Regardless of the fact that in

row 2 the water came successively in contact with more diseased plants as it flowed from section to section, in July of the second year little difference was found in the percentage of dwarf in any of the four sections of plants grown from seed. In row 1, out of 248 plants grown from seed, 24, or approximately 10 percent, were diseased. The distribution of the disease in these two rows, together with that in the four adjacent rows, indicates that the water was not the principal agent involved in carrying the causal agent.

Additional evidence that water is not the important agent in distributing dwarf in the field was obtained by preparing a seedbed about 3 feet wide by 100 feet long in such a way that the top of the bed was several inches higher than the furrows in which the irrigation water ran. The elevated seedbed and the furrows were seeded with common alfalfa on March 12, 1930. The plants on the raised bed were able to get sufficient moisture without the water coming in contact with the crowns. Of course the winter rains could not be excluded, but since the bed was elevated and a furrow was present on either side, the water ran over the surface only a very short distance. On November 18, 1932, there were 30 percent of dwarfed plants on the raised bed and 22 percent in the furrows, showing that the disease was equally as serious where the irrigation water did not come in contact with the tops or crowns of the plants as where it did. This was considered important, as dwarf can be detected first in the crowns.

DISEASED TISSUE

Since the disease did not appear to be soil-borne, but was transmissible from plant to plant, it seemed desirable to ascertain whether plants growing in soil in which pieces of diseased tissue were present would take the disease. The results of experiments designed to determine this point are shown in table 3.

Soil from about badly diseased plants was placed in a pot and diseased roots cut in short lengths were added; Hairy Peruvian alfalfa was sown in it on April 4, 1929. On April 28, 1931, out of 17 plants, 9, or 53 percent, had dwarf. Control plants growing in mountain soil remained healthy.

TABLE 3.—*Transmission of dwarf by means of diseased tissue in the soil*

Date plants set out	Date final notes taken	Plants inoculated	Control plants	Plants infected				Part of plant tissue used
				Inoculated		Control		
		Number	*Number*	*Number*	*Percent*	*Number*	*Percent*	
Apr. 4, 1929	Apr. 28, 1931	17	4	9	53	0	0	Roots.
Sept. 12, 1932	Aug. 3, 1934	25	25	0	0	0	0	Stems.
Do	do	25	25	4	16	0	0	Roots.
May 16, 1934	May 28, 1935	14		0	0	0	0	Stems and roots.

On September 12, 1932, a quantity of stems from badly diseased plants were cut into small pieces and mixed into the surface 2 inches of soil of a 4- by 4-foot basin. A control was prepared in the same manner except that healthy stems were used. Thinly sliced diseased roots were mixed into the soil of a third basin, and a fourth was left

untreated. Each of the four basins was planted with 25 young healthy alfalfa seedlings. On August 3, 1934, no disease was found in the basins to which the stems had been added or in the untreated basin, but in the basin to which the diseased roots had been added there were four diseased plants.

Although too few to be conclusive, these experiments suggest that dwarf may be carried in the soil in pieces of root tissue but probably not in the green stems. Just how the causal agent gets from the segments of diseased roots into the roots of the healthy plants under these conditions is not known, unless it is carried by some soil insect.

On May 16, 1934, 14 healthy plants were set in cans in mountain soil and the roots were surrounded by a quart of diseased root and stem tissue that had been passed through a food grinder. No disease had developed by May 28, 1935. Seemingly every opportunity for infection was offered had there been a causal agent present that was capable of penetrating the roots directly or through wounds.

Since the disease was not carried by the diseased stems in one of the experiments reported above, it seems probable that the disease is not carried in the hay or seed. This is a single experiment, however, and should not be given too much weight, although the results are apparently confirmed by the experiments with stem tissue and juice, which are described later in this paper.

CUTTINGS

Although the stems of diseased alfalfa plants when added to the soil did not transmit dwarf to healthy plants, the possibility that the causal agent is in the stems was not entirely excluded. In order to determine more definitely whether the disease is present in the green tops, the experiments recorded in table 4 were conducted.

Two sets of cuttings were made from stems of dwarfed plants on February 17, 1930. In one set some of the crown tissue was included and in the other only green tissue was used. These cuttings were rooted in sand and later planted in cans of mountain soil. Several cans of the same soil containing 100 healthy plants grown from seed were used as controls to check on possible outside sources of infection. On August 18, 1931, of the plants grown from cuttings from green stems alone, only four had dwarf, four remained healthy, and two had died from some unknown cause, possibly dwarf; whereas of the plants grown from cuttings that included some of the crown tissue, eight had dwarf, one was healthy, and one had died of crown rot. A higher percentage of infection in the latter group suggests that the disease was more consistently present in the bases of the stems and in the crown tissue than in the green stems. No disease developed in any of the controls.

On December 30, 1932, cuttings were made from the upper ends of the stems of plants in an early stage of dwarf, where it seemed very improbable that any of the bacterialike bodies or gum in their vessels would be found, especially as the stems had grown late in the season, when dwarf is inactive. Comparable cuttings were made from healthy stems as controls. The cuttings were rooted in sand and transplanted to soil in cans. On July 20, 1934 the plants were examined, and 15 out of 43, or 35 percent, of those from diseased plants had dwarf. The remainder, as well as the controls, were healthy.

TABLE 4.—*Transmission of dwarf in cuttings from diseased stems*

Date cuttings made	Date final notes taken	Cuttings		Plants infected			
		Healthy control	Diseased	From healthy plants		From diseased plants	
		Number	*Number*	*Number*	*Percent*	*Number*	*Percent*
Feb. 17, 1930	Aug. 18, 1931	[1] 50	[2] 10	0	0	4	40
Dec. 30, 1932	July 20, 1934	[1] 50	[3] 10	0	0	8	80
		50	43	0	0	15	35

[1] Plants grown from seed. [2] Cuttings from green stems alone. [3] Cuttings included some crown tissue.

This experiment, like the one previously described, shows that the causal agent is present in at least part of the green stems of dwarfed plants. All of these cuttings were made during the winter when the dwarf was either entirely inactive or nearly so. Many cuttings were made at other times but none grew, hence only results from cuttings made in the winter are available.

LEAF MUTILATION AND PINPRICK INOCULATION

As already shown, the dwarf disease is present even during the winter in at least a part of the green stems. Attempts were made, therefore, to transmit dwarf by certain more or less standard methods used by investigators of virus diseases.

Two methods of leaf mutilation were tried. In the first, leaves of diseased and then of healthy plants were crushed between the thumb and fingers, and in the second, healthy and diseased leaves and stems were crushed together. One hundred plants were thus treated, but no disease resulted.

In another experiment 12 micro-insect pins, pushed through a piece of cardboard to hold them in position, were used to prick juice from diseased into healthy alfalfa leaves. Terminal leaves of diseased and healthy plants were held in contact with one another and pricked repeatedly. A number of leaves on 27 plants were inoculated in this manner, but no disease developed in any of them.

JUICE

Several attempts were made to transmit dwarf by inserting juice from dwarfed tops and roots into healthy plants. Juice, obtained by macerating the tissue in a food grinder and squeezing it by hand through cheesecloth, was injected into the healthy plants as soon as possible after extraction, each plant usually being subjected to several injections. Controls in which juice from healthy plants or sterile distilled water replaced the juice from diseased plants were prepared in some cases. Cotton dipped into the juice of diseased plants was inserted into the root or the base of the stems of healthy plants and allowed to remain. In other cases juice was injected into healthy roots with a hypodermic needle or applied to freshly cut stems with a pipette dropper. None of the 95 inoculated or the 95 control plants became infected. That dwarf is not transmitted, at least not readily, by means of diseased juice applied mechanically was concluded.

TISSUE INOCULATION

Experiments were conducted to determine whether dwarf could be transmitted by inserting bits of diseased tissue into healthy plants.

For the most part this type of experiment consisted in making an opening into the healthy plant by means of a scalpel, inserting the diseased tissue, and either leaving the wound open or, more often, closing it as well as possible with tape, raffia, paraffin, or tree seal. In many cases the inoculum consisted of a small piece of yellow wood, about 15 to 20 mm long, 1 to 2 mm thick, and 5 to 10 mm wide, taken from a diseased taproot after the bark was removed. Sometimes the tissue was macerated with a mortar and pestle before it was inserted into the wound. Pieces of stems, rootlets, leaves, buds, bark from the taproot, and white wood from diseased roots were tried as sources of inoculum. An attempt was made in many cases to crush the diseased tissue after it had been placed in the wound, so that some of the juice from the diseased tissue actually might be mixed with that of the healthy wood. Comparable controls were run in most cases.

Table 5 shows that out of a total of 1,114 plants inoculated 63 plants, or 6 percent, became infected. Of the 409 controls, only 1, or 0.24 percent, developed the disease. Of 672 plants, infection resulted in 63, or 9 percent, when yellow wood was used as the inoculum; the number infected included the 1 plant inoculated with crushed rootlets. Only in the experiment initiated July 12, 1932, with yellow wood, was there a high percentage of infection, namely, 84 percent. Why such a high percentage of infection was obtained in this experiment is not known. Infection resulted only in those experiments in which yellow wood was used as the inoculum.

TABLE 5.—*Transmission of alfalfa dwarf by inserting diseased tissue into healthy plants*

Inoculum used	Date inoculated	Date final notes taken	Plants inoculated	Control plants	Plants infected			
					Inoculated		Control	
			Number	*Number*	*Number*	*Percent*	*Number*	*Percent*
Yellow xylem	June 7, 1930	Aug. 19, 1931	5	0	0	0		
Do	Aug. 12, 1930	July 3, 1931	10	10	1	10	0	0
Do	Nov. 7, 1930	Nov. 19, 1931	10	0	0	0	0	0
Do	Dec. 5, 1930	Sept. 3, 1931	50	50	15	30	1	2
Do	June 15, 1931	Sept. 6, 1932	25	[1] 50	0	0	0	0
Green stems	do	do	50		0	0	0	0
Leaves	do	do	25		0	0	0	0
Crushed yellow xylem	do	do	25		3	12	0	0
Crushed bark	do	do	25		0	0	0	0
Crushed rootlets	do	do	25		1	4	0	0
Yellow xylem	Apr. 8, 1932	June 23, 1933	43	25	0	0	0	0
Do	May 9, 1932	do	44	25	0	0	0	0
Do	June 17, 1932	do	45	25	0	0	0	0
Do	July 11, 1932	do	46	25	1	2	0	0
Do	Aug. 26, 1932	do	49	25	0	0	0	0
Do	July 12, 1932	Nov. 29, 1932	50	24	42	84	0	0
Green stems	do	do	50	25	0	0	0	0
White xylem from diseased plants.	do	do	50	25	0	0	0	0
Yellow xylem	Jan. 5, 1933	June 22, 1933	100	25	0	0	0	0
White wood from diseased root.	June 6, 1934	Sept. 21, 1934	34	10	0	0	0	0
White bark from diseased root.	do	do	52	35	0	0	0	0
Yellow xylem	do	do	145	10	0	0	0	0
Green stems	do	do	54	10	0	0	0	0
Terminal leaves	do	do	57	10	0	0	0	0
Bark over yellow wood	do	do	35	0	0	0	0	0
Total			1,104	409	63	[2] 6	1	[2] .24

[1] These 50 plants served as a control for all of the inoculations made on June 15, 1931.
[2] Average percent.

APPROACH GRAFTING

Since grafting has been found to be one of the most successful methods of transmitting virus diseases that are not readily transmitted mechanically, a type of approach grafting was tried. This consisted in cutting away a portion of the bark and wood of the taproots of a diseased and of a healthy plant, placing the wounded surfaces together, and fastening them in that position by means of rubber electric tape or raffia (fig. 1). The plants were then set in

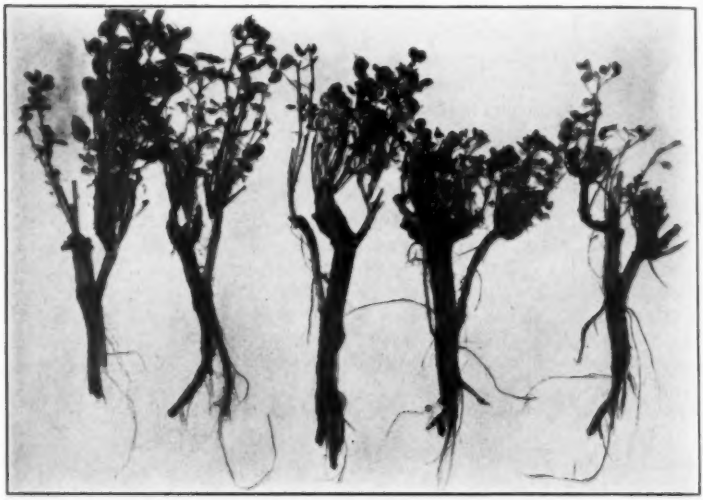

FIGURE 1.—Approach grafting of diseased and healthy alfalfa plants. The larger roots were diseased. The smaller roots were healthy when grafted but were diseased when photographed, although top symptoms were not yet conspicuous. About one-fifth natural size.

TABLE 6.—*Transmission of alfalfa dwarf by approach grafting*

Date grafted	Date final notes taken	Plants grafted	Control plants	Plants infected [1]	
		Number	*Number*	*Number*	*Percent*
May 8, 1930	Aug. 18, 1931	3	0	2	67
Nov. 7, 1930	Nov. 19, 1931	4	0	3	75
July 15, 1931	Sept. 2, 1932	25	0	8	32
Apr. 8, 1932	June 23, 1933	45	25	13	29
May 24, 1932	do	39	25	25	64
June 17, 1932	do	33	25	19	58
July 11, 1932	do	33	25	7	21
Aug. 25, 1932	do	50	25	8	16
May 25, 1932	do	50	0	7	14
July 15, 1931	Sept. 2, 1932	25	25	8	32
Mar. 1, 1934	Aug. 1, 1934	20	25	3	15
Do	do	23	25	10	43
Do	do	26	25	7	27
Do	do	91	25	28	31
May 16, 1934	May 28, 1935	8	[2] 8	3	38
June 13, 1934	Sept. 17, 1934	35	0	11	31
Total		510	258	162	[3] 37

[1] None of the control plants became infected.
[2] Entire diseased plants were killed by boiling in water and were then grafted to healthy roots.
[3] Average percent.

cans or basins and allowed to grow, usually for several months, before they were examined. The results of a number of such experiments are recorded in table 6. Controls were prepared by grafting two healthy plants. In the experiment set up on May 16, 1934, the control consisted of diseased plants that were killed by being boiled in water before they were grafted on healthy plants. A total of 510 plants were grafted, of which 162, or 32 percent, became diseased. No disease developed in any of the 258 controls.

During the spring and summer of 1932 grafts were made in April, May, June, July, and August, in order to study seasonal influences. In this experiment, as shown in table 6, the highest amount of infection developed in the plants grafted early in the season, namely, in April, May, and June. This was probably due, in part at least, to the fact that earlier in the season the plants recovered more quickly and completely from the shock of transplanting, and possibly also to more rapid and extensive union of the diseased and healthy tissues. On the other hand, the lowest percentage of infection obtained resulted from the inoculations made on May 25, 1932. Although the infection that developed in these 16 experiments averaged only 37 percent, it is evident that the dwarf disease can be transmitted by approach grafting. In many cases no union or only a very poor one was formed between the diseased and healthy plants; in others there was a fair union at the upper parts of the wounds, although usually the amount of tissue grown together was not large. The evidence indicates that a definite union is necessary, at least for a high percentage of infection. In a few instances no union was apparent at the time the plants were examined, and yet transmission had taken place. Apparently only a slight union is necessary, and it is possible that occasionally transmission takes place without a union.

OTHER GRAFTING METHODS

Other methods of budding and grafting were tried in an effort to find a more consistent means of transmitting dwarf. Scions consisting of part crown and part stem tissue and buds taken from the crowns of diseased plants were inserted into the crown branches of healthy plants by the cleft-graft method, but they failed to grow, and no disease resulted.

The results of three other types of grafting are shown in table 7. In the first two experiments a piece of diseased taproot about 3 inches long was grafted on the taproot of a healthy plant about 6 inches below the crown by the ordinary cleft-graft method (fig. 2). The cleft was made in the old root, and, after insertion of the wedge-shaped end of the healthy root, the graft was wrapped tightly with raffia and covered with tree seal. Figure 3 shows that a high percentage of infection was obtained. A union was formed in all but 1 of the 31 plants inoculated in these two experiments, and infection resulted in all but this 1 plant. Roots grafted in this manner formed a union more consistently and made a better union than was done by any other method tried.

On June 15, 1931, the ends of a healthy and of a diseased root were grafted, the end of the diseased root, about 6 inches below the crown, being used as the stock and that of the healthy root as the scion. A considerably lower percentage of infection resulted (35 percent), but

there is no doubt that infection can be obtained by this method. The grafts in the last experiment in table 7 were made in a similar manner except that a slit was made entirely through the diseased root, through

FIGURE 2.—Healthy control alfalfa plant at left. Three plants at right are from same lot of plants as the control, but are dwarfed as a result of grafting a piece of diseased root to their taproot. Grafted June 7, 1934, and photographed September 9, 1934. About one-sixth natural size.

which the healthy root was inserted, so that the two crowns were at a common level. The bark was removed from the healthy root at the point of contact with the diseased one. This was similar to an insert

graft except that the healthy root passed entirely through the diseased root and the cambiums made contact on both sides of the roots. These experiments indicate that dwarf can be transmitted by any method of grafting in which a union is formed.

TABLE 7.—*Transmission of dwarf by other grafting methods*

Date grafted	Date final notes taken	Plants grafted	Control plants	Plants infected [1]	
		Number	*Number*	*Number*	*Percent*
June 7, 1934 [2]	Sept. 18, 1934	21	25	21	100
June 5, 1934 [3]	Sept. 21, 1934	10	10	9	90
June 15, 1931 [3]	Sept. 2, 1932	23	25	8	35
Mar. 1, 1934 [4]	Sept. 21, 1934	18	25	10	56

[1] None of the control plants became infected.
[2] Section of diseased root grafted on the end of a healthy root.
[3] Ends of healthy and diseased roots grafted together.
[4] Healthy root inserted through a slit in the diseased root.

FIGURE 3.—Healthy control alfalfa plants at extreme right. Other plants show typical dwarf resulting from grafting by method illustrated in figure 2. One-hundred-percent infection was obtained in this experiment. Grafted June 7, 1934, and photographed September 9, 1934.

DISCUSSION

The presence of bacterialike bodies in the ducts of the roots, crowns, and bases of the stems of dwarfed alfalfa plants, even in very early stages of the disease, at first created the impression that they must be the causal agents. Isolations showed that not one but many different bacteria were present in the tissue, none of which would reproduce the disease under the experimental conditions provided.

The top symptoms of alfalfa plants affected with dwarf, such as the darker green foliage, are not unlike those of other plants affected with certain virus diseases; for example, phony disease of peach (*5*), spindle tuber and curly dwarf of potato (*4*), dwarf disease of rice (*3*), and a dwarf disease of sugarcane (*2*).

The presence of so much yellowing in the wood of the root and the plugging of the vessels with gum in which the bacterialike bodies are embedded constitute the chief differences between the dwarf disease of alfalfa and most other virus diseases. The presence of gum in the xylem vessels of plants affected with virus diseases is not unknown. Xylem vessels of potato plants affected with top necrosis may become filled with gum (*1*). Some plugging of vessels in the roots of lettuce (*Lactuca sativa* L.) plants affected with spotted wilt have been observed. Likewise a brown discoloration in the vascular system of lupine has been described (*7*).

Not only is the plugging of the xylem vessels with gum known to occur in the case of some virus diseases, but bacteria also have been found closely associated with some virus-affected plants (*6*, *7*, *8*, *9*).

Clearly, then, the presence of gum in the xylem ducts and the association of bacteria and bacterialike bodies with the diseased tissue are not sufficient to exclude alfalfa dwarf from the group of virus diseases as at present constituted.

SUMMARY AND CONCLUSIONS

The data presented show that alfalfa dwarf is not due to an unfavorable soil condition and is not caused by a fungus. The possibility that dwarf is due to a bacterium may not be entirely excluded; however, all of the evidence obtained argues against such a conclusion. Bacteria are present in considerable abundance in at least 75 percent of the infected roots, but none of those isolated caused infection under the conditions of the experiments.

The causal agent of dwarf does not live in soil free of diseased plants. Pieces of diseased roots added to the soil appeared to serve as a source of inoculum in some experiments, but stems from diseased plants did not.

Dwarf spreads readily from diseased to nearby healthy plants.

Water in itself is not an important factor in disseminating dwarf, although it might wash out and transport diseased living root tissue or possibly carry an insect vector.

Cuttings of the stems of diseased plants contained the causal agent and produced from 35 to 80 percent of diseased plants.

Leaf mutilation and pinprick methods of inoculation gave negative results. Likewise, juice inoculations failed to give infection.

Inserting bits of tissue from diseased plants into wounds in healthy plants failed to produce infection except when yellow wood of the root or crown was used. Even then the percentage of infection was very small except in one experiment in which 84-percent infection was obtained. Out of a total of 672 plants inoculated with yellow xylem 63, or 9 percent, became diseased.

Any method of grafting in which diseased and healthy root tissue formed a union gave a high percentage of transmission. One hundred-percent transmission was obtained in one experiment and 90 percent in another by grafting a piece of diseased root onto the lower end of the taproot of the healthy plant. This method gave the highest percentage of infection of any tried, presumably because a better union was formed.

The evidence presented seems to show that alfalfa dwarf is a virus disease belonging to the group that can be transmitted by grafting, but not by juice inoculation.

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NUTRITIVE VALUE OF THE PROTEIN IN CALF LUNGS, COW UDDERS, AND HOG SPLEENS[1]

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INTRODUCTION

In the commercial slaughter of meat food animals in the United States, the various meat byproducts are utilized in the manufacture of meat food products and animal feed. The nutritive value of meat byproducts is naturally of considerable interest both to the meat packer and to the consumer. In previous papers the writers have reported the nutritive value of the protein in a number of meat byproducts. The present paper gives similar information for calf lungs, cow udders, and hog spleens, together with some data on cracklings. These products, although used only to a limited extent for human food, are extensively employed in the manufacture of animal feed. The purpose of the experiments here reported was to determine the nutritive value of the protein in each meat byproduct as compared with that of the protein in lean beef, and the value of the proteins in the several animal tissues as supplements for each other and for the protein in corn meal.

PREVIOUS INVESTIGATIONS

The protein in beef spleen was found by Hoagland and Snider [2] to have practically the same value for maintenance and growth in young rats as the protein in veal, ox and hog brains, ox and hog tongues, and dried milk. In a later paper these authors[3] reported that the protein in calf lungs had an appreciably lower nutritive value than that in veal. Cracklings were found to contain protein of very low nutritive value, since 15 percent of protein from this source was required for maintenance in rats.

The protein in beef was found by Hoagland and Snider [4] to have a marked supplemental relationship for the protein in corn meal, wheat, bolted wheat flour, and oatmeal. When one part of beef protein was fed in a mixture with two parts of any one of the vegetable proteins, the mixture had the same value for maintenance and growth as a like quantity of beef protein alone.

Mitchell and Carman [5] found that the protein in both beef and veal supplemented the protein in patent white flour. When two parts of white-flour protein was fed in combination with one part

[1] Received for publication Feb. 18, 1936; issued October 1936.

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of beef or veal protein, the protein in the mixture had as high a value as that in beef alone.

EXPERIMENTAL PROCEDURE

Hog spleens, calf lungs, and beef round were purchased from local meat-packing establishments, and cow udders and cracklings were obtained from Chicago. Federal meat inspection was maintained at these establishments, and the products purchased had been inspected and passed for food purposes. Each meat byproduct was trimmed free of extraneous tissue, ground, and dried in an oven in a current of air at a temperature not exceeding 60° C. The dried products were ground fine and stored at approximately 2° C. until their nutritive value was tested by feeding to young, male, albino rats.

Each lot of the dried tissue was analyzed for nitrogen and fat. Each feed mixture was then made up to contain 8 percent of protein ($N \times 6.25$) derived from the product or products to be tested. In addition, approximately 0.9 percent of protein was introduced in the 2 percent of yeast concentrate added as a source of vitamins B and G. One percent of cod-liver oil supplied vitamins A and D. The fat content of each mixture was adjusted to 4.4 percent by the addition of a sufficient quantity of lard. Four percent of ash and sufficient cassava starch to make 100 percent comprised the remainder of the mixture. When needed, each mixture was made up in the quantity of 1,000 g and was stored in a tightly covered glass jar for use during the experiment.

The rats used in these experiments were selected from a colony of albino rats which has been maintained for a number of years by the Bureau's biochemic laboratory. Litters of more than eight rats were reduced to that number within a few days after birth. Only male rats that weighed at least 40 g within 30 days after birth were used. Each group of rats fed a particular ration was selected from four or more litters.

Each ration was fed to a group of from five to eight rats for 60 days, and the experimental data are tabulated both for the first 30 days and for the entire period. Each rat was kept in a cylindrical wire cage with a raised screen bottom. The ration was supplied in a self-feeder. The rats were weighed twice weekly at regular intervals, and records were kept of the feed consumed.

RESULTS OF EXPERIMENTS

The results of the feeding experiments are presented in table 1. In judging the relative nutritive value of the protein in the different products, the average gain in weight per gram of protein consumed by each group of rats is of particular significance. Those experiments in which only one product was the main source of protein in the ration indicate that beef had the highest value, followed closely by calf lungs and hog spleens; cow udders and corn meal had much lower values. The nutritive value of cracklings as the main source of protein in the diet was not determined in these experiments since the writers [6] had previously found the protein in this product to be of very poor quality.

[6] HOAGLAND, R., and SNIDER, G. G. See footnote 3.

TABLE 1.—*Comparative nutritive values of the proteins in various meats and meat byproducts fed singly, in combination among themselves, or with corn meal, to young male, albino rats*

Source of protein tested	Rat no.	Age of rats at beginning of test	Initial weight of rats	Gains and intake of rats in 30 days						Gains and intake of rats in 60 days					
				Total gain in weight	Total intake of—		Intake, per gram gain in weight, of—		Gain, in weight, per gram of—	Total gain in weight	Total intake of—		Intake, per gram gain in weight, of—		Gain, in weight, per gram of—
					Feed	Protein	Feed	Protein	Protein		Feed	Protein	Feed	Protein	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Calf lungs	1244A	23	42	81	315	25.2	3.9	0.31	3.21	149	660	52.8	4.4	0.35	2.82
	1244B	23	43	83	320	25.6	3.9	.31	3.24	171	740	59.2	4.3	.35	2.89
	1244C	23	43	94	344	27.5	3.7	.29	3.42	183	792	63.4	4.3	.35	2.89
	1244D	23	40	79	322	25.8	4.1	.33	3.06	149	708	56.6	4.8	.38	2.63
	1244E	22	43	80	344	27.5	4.3	.34	2.91	156	758	60.6	4.8	.39	2.57
	1244F	24	41	74	312	25.0	4.2	.34	2.96	155	720	57.6	4.6	.37	2.69
Average		23	42	82	326	26.1	4.0	.32	3.13	161	730	58.4	4.6	.37	2.75
Calf lungs and yellow corn meal [1]	1245A	26	40	76	313	25.0	4.1	.33	3.04	153	731	58.5	4.8	.38	2.62
	1245B	24	44	111	403	32.2	3.6	.29	3.45	215	902	72.2	4.2	.34	2.98
	1245C	23	40	89	337	27.0	3.8	.30	3.30	190	796	63.7	4.2	.34	2.98
	1245D	24	40	110	389	31.1	3.5	.28	3.54	229	896	71.7	3.9	.31	3.19
	1245E	23	43	112	398	31.8	3.5	.28	3.52	223	914	73.1	4.1	.33	3.05
Average		24	41	100	368	29.4	3.7	.30	3.37	202	848	67.8	4.2	.34	2.96
Hog spleens	1248A	22	45	68	263	21.0	3.9	.31	3.24	108	543	43.4	5.0	.40	2.49
	1248B	24	45	99	371	29.7	3.7	.30	3.33	209	897	71.8	4.3	.34	2.91
	1248C	25	42	69	290	23.2	4.2	.34	2.97	122	626	50.1	5.1	.41	2.44
	1248D	21	37	86	343	27.4	4.0	.32	3.14	176	811	64.9	4.6	.37	2.71
	1248E	21	44	76	332	26.6	4.4	.35	2.86	148	753	60.2	5.1	.41	2.46
	1248F	25	47	81	350	28.0	4.3	.35	2.89	162	777	62.2	4.8	.38	2.60
Average		23	43	80	325	26.0	4.1	.33	3.07	154	735	58.8	4.8	.39	2.60
Hog spleens and yellow corn meal [1]	1249A	28	44	108	381	30.5	3.5	.28	3.54	163	773	61.8	4.7	.38	2.64
	1249B	21	40	119	421	33.7	3.5	.28	3.53	224	952	76.2	4.3	.34	2.94
	1249C	21	40	94	355	28.4	3.8	.30	3.31	196	844	67.5	4.3	.34	2.90
	1249D	25	46	132	448	35.8	3.4	.27	3.69	264	1,009	80.7	3.8	.31	3.27
	1249E	24	44	134	456	36.5	3.4	.27	3.67	234	1,067	85.4	4.6	.36	2.74
	1249F	24	43	114	428	34.2	3.8	.30	3.33	211	937	75.0	4.4	.36	2.81
Average		24	43	117	415	33.2	3.6	.28	3.51	215	930	74.4	4.4	.35	2.88

[1] 14 percent of protein from each product.

TABLE 1.—*Comparative nutritive values of the proteins in various meats and meat byproducts fed singly, in combination among themselves or with corn meal, to young male, albino rats*—Continued

Source of protein tested	Rat no.	Age of rats at beginning of test	Initial weight of rats	Gains and intake of rats in 30 days						Gains and intake of rats in 60 days						
				Total gain in weight	Total intake of—		Intake, per gram gain in weight, of—		Gain, in weight, per gram of—		Total gain in weight	Total intake of—		Intake, per gram gain in weight, of—		Gain, in weight, per gram of—	
					Feed	Protein	Feed	Protein	Feed	Protein		Feed	Protein	Feed	Protein	Feed	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Cow udders	1268A	24	46	70	313	25.0	4.5	.36	.22	2.80	116	671	53.7	5.8	.46	.17	2.16
	1268B	19	44	81	336	26.9	4.1	.33	.24	3.01	152	784	62.7	5.2	.41	.19	2.42
	1268C	20	40	62	295	23.6	4.8	.38	.21	2.63	129	720	57.6	5.6	.45	.18	2.24
	1268D	22	40	62	296	23.7	4.8	.38	.21	2.62	116	663	53.0	5.7	.46	.17	2.19
	1268E	23	47	45	263	21.0	5.8	.47	.17	2.14	90	586	46.9	6.5	.52	.15	1.92
	1268F	23	39	67	283	22.6	4.2	.34	.24	2.96	119	657	52.6	5.5	.44	.18	2.26
	1268G	27	40	49	217	17.4	4.4	.36	.23	2.82	90	499	39.9	5.5	.44	.18	2.26
	1268H	20	41	39	208	16.6	5.3	.43	.19	2.35	78	452	36.2	5.8	.46	.17	2.15
Average		22	42	59	276	22.1	4.7	.38	.21	2.67	111	629	50.3	5.7	.46	.17	2.20
Cow udders and yellow corn meal [1]	1269A	25	43	51	249	19.9	4.9	.39	.20	2.56	92	553	44.2	6.0	.48	.17	2.08
	1269B	20	42	53	263	21.0	5.0	.40	.20	2.52	126	638	51.0	5.1	.40	.20	2.47
	1269C	21	43	68	305	24.4	4.5	.36	.22	2.79	166	775	62.0	4.7	.37	.21	2.68
	1269D	23	40	56	283	22.6	5.1	.40	.20	2.48	112	644	51.5	5.8	.46	.17	2.17
	1269E	25	46	50	275	22.0	5.5	.44	.19	2.27	116	647	51.8	5.6	.45	.18	2.24
	1269F	20	41	50	222	17.8	4.4	.36	.23	2.81	106	503	40.2	4.7	.38	.21	2.64
	1269G	21	42	53	262	21.0	4.9	.40	.20	2.52	107	570	45.6	5.3	.43	.19	2.35
	1269H	22	42	77	345	27.6	4.5	.36	.22	2.79	156	747	59.8	4.8	.38	.21	2.61
Average		22	42	57	276	22.0	4.9	.39	.21	2.59	123	635	50.8	5.3	.42	.19	2.41
Hog spleens, calf lungs, cow udders, and cracklings [2]	1270A	20	42	51	208	16.6	4.1	.33	.25	3.07	88	449	35.9	5.1	.41	.20	2.45
	1270B	25	44	48	274	21.9	5.7	.46	.18	2.19	97	573	45.8	5.9	.47	.17	2.12
	1270C	21	42	66	301	24.1	4.6	.37	.22	2.74	127	664	53.1	5.2	.42	.19	2.39
	1270D	21	48	59	296	23.7	5.0	.40	.20	2.49	106	621	49.7	5.9	.47	.17	2.13
	1270E	25	46	68	331	26.5	4.9	.39	.21	2.57	136	696	55.7	5.1	.41	.20	2.44
	1270F	24	45	74	234	18.7	3.2	.25	.32	3.96	136	593	47.4	4.4	.35	.23	2.87
	1270G	31	43	42	216	17.3	5.1	.41	.19	2.43	73	463	37.0	6.3	.51	.16	1.97
	1270H	22	44	44	182	14.6	4.1	.33	.24	3.01	82	407	32.6	5.0	.40	.20	2.52
Average		24	44	57	255	20.4	4.6	.37	.23	2.81	106	558	44.7	5.4	.43	.19	2.36

Hog spleens, calf lungs, cow udders, cracklings, and yellow corn meal [3]	1271A	28	42	33	180	14.4	5.5	.44	.18	2.29	79	420	33.6	5.3	.43	.19	2.35
	1271B	22	43	42	176	14.1	4.2	.34	.24	2.98	88	393	31.4	4.5	.36	.22	2.80
	1271C	25	43	42	235	18.8	5.6	.45	.18	2.23	85	486	38.9	5.7	.46	.17	2.19
	1271D	26	39	40	225	18.0	5.6	.45	.18	2.22	77	468	37.4	6.1	.49	.16	2.06
	1271E	22	39	36	154	12.3	4.3	.34	.23	2.93	84	366	29.3	4.4	.35	.23	2.87
	1271F	23	41	43	182	14.6	4.2	.34	.24	2.95	91	412	33.0	4.5	.36	.22	2.76
	1271G	25	42	47	209	16.7	4.4	.36	.22	2.81	114	488	39.0	4.3	.34	.23	2.92
	1271H	25	47	36	169	13.5	4.7	.38	.21	2.67	89	406	32.5	4.6	.37	.22	2.74
Average		25	42	40	191	15.3	4.8	.39	.21	2.64	88	430	34.4	4.9	.40	.21	2.59
Beef round	1256A	22	46	90	366	29.3	4.1	.33	.25	3.07	190	824	65.9	4.3	.35	.23	2.88
	1256B	23	45	95	358	28.6	3.8	.30	.27	3.32	209	951	76.1	4.6	.36	.22	2.75
	1256C	22	40	90	328	26.2	3.6	.29	.27	3.44	183	764	61.1	4.2	.33	.24	3.00
	1256D	22	43	91	349	27.9	3.8	.31	.26	3.26	178	796	63.7	4.5	.36	.22	2.79
	1256E	23	41	72	294	23.5	4.1	.33	.24	3.06	141	666	53.3	4.7	.38	.21	2.65
	1256F	25	43	97	354	28.3	3.6	.29	.27	3.43	177	668	53.4	4.8	.30	.27	3.31
	1256G	22	40	92	344	27.5	3.7	.30	.27	3.35	165	754	60.3	4.6	.37	.22	2.74
	1256H	23	40	86	337	27.0	3.9	.31	.26	3.19	152	705	56.4	4.6	.37	.22	2.70
Average		23	42	89	341	27.3	3.8	.31	.26	3.27	174	766	61.3	4.4	.35	.23	2.85
Beef round and yellow corn meal [1]	1257A	23	42	98	364	29.1	3.7	.30	.27	3.37	191	821	65.7	4.3	.34	.23	2.91
	1257B	24	40	100	368	29.4	3.7	.29	.27	3.40	221	885	70.8	4.0	.32	.25	3.12
	1257C	23	41	82	298	23.8	3.6	.29	.28	3.45	182	735	58.8	4.0	.32	.25	3.10
	1257D	21	44	109	388	31.0	3.6	.28	.28	3.52	226	887	71.0	3.9	.31	.25	3.18
	1257E	24	41	93	347	27.8	3.7	.30	.27	3.35	185	789	63.1	4.3	.34	.23	2.93
	1257F	25	40	84	326	26.1	3.9	.31	.26	3.22	197	792	63.4	4.0	.32	.25	3.11
	1257G	24	46	104	392	31.4	3.8	.30	.27	3.31	226	927	74.2	4.1	.33	.24	3.05
	1257H	21	39	79	325	26.0	4.1	.33	.24	3.04	178	708	56.6	4.0	.32	.25	3.14
Average		23	42	94	351	28.1	3.8	.30	.27	3.33	201	818	65.5	4.1	.33	.24	3.07
Yellow corn meal	1243A	22	45	56	315	25.2	5.6	.45	.18	2.22	121	712	57.0	5.9	.47	.17	2.12
	1243B	22	45	60	322	25.8	5.4	.43	.19	2.33	131	748	59.8	5.7	.46	.18	2.19
	1243C	23	41	48	276	22.1	5.8	.46	.17	2.17	104	635	50.8	6.1	.49	.16	2.05
	1243D	21	41	55	290	23.2	5.3	.42	.19	2.37	124	679	54.3	5.5	.44	.18	2.28
	1243E	23	42	60	319	25.5	5.3	.43	.19	2.35	126	731	58.5	5.8	.46	.17	2.15
	1243F	23	41	51	289	23.1	5.7	.45	.18	2.21	114	668	53.4	5.9	.47	.17	2.13
	1250A	19	42	47	262	21.0	5.6	.45	.18	2.24	72	550	44.0	7.6	.61	.13	1.64
	1250B	22	40	47	264	21.1	5.6	.45	.18	2.23	89	590	47.2	6.6	.53	.15	1.89
	1250C	21	39	48	300	24.0	6.3	.50	.16	2.00	91	623	49.8	6.8	.55	.15	1.83
	1250D	21	40	39	263	21.0	6.7	.54	.15	1.86	86	600	48.0	7.0	.56	.14	1.79
	1250E	25	46	66	344	27.5	5.2	.42	.19	2.40	128	741	59.3	5.8	.46	.17	2.16
	1250F	24	46	65	350	28.0	5.4	.43	.19	2.32	133	780	62.4	5.9	.47	.17	2.13
Average		22	42	54	300	24.0	5.7	.45	.18	2.23	110	671	53.7	6.2	.50	.16	2.03

[1] 4 percent of protein from each product. [2] 2 percent of protein from each product. [3] 1 percent of protein from first 4 products; 4 percent from yellow corn meal.

The experiments in which the rations contained mixtures of animal products and corn meal are of particular interest because in several of them the results indicate a marked supplementary relationship between the two kinds of protein. Two proteins may be said to supplement each other if a mixture of the two has a higher biological value than their calculated combined value based on the values determined for the proteins individually and the proportions in which they were combined. Obviously the proteins supplement each other if the mixture has as high or higher nutritive value than the better of the two proteins. In making such comparisons all proteins must have been fed at the same level of intake.

In calculating the probable biological value of the protein in a mixture of an animal product and corn meal, assuming that no supplementary relationship exists, the writers have used essentially the method followed by Mitchell and Carman.[7] For example, table 1 shows that the feeding of calf lungs resulted in an average gain in weight in 60 days of 2.75 g for each gram of protein consumed, corn meal a gain of 2.03 g, and a mixture of equal parts of lung and corn protein a gain of 2.96 g. If the two kinds of protein did not supplement each other, then the calculated value for the mixture would be $\frac{2.75+2.03}{2}=2.39$ g. However, since the mixture of lung and corn proteins actually brought about a gain of 2.96 g for each gram of protein consumed, or an increase of 0.57 g over the calculated value, there was evidently a material supplementary relationship between the two kinds of protein. Furthermore, the animals made materially higher gains when fed the mixture of proteins, both for the 30- and the 60-day tests, than when fed lung protein alone.

The protein in hog spleens supplemented that in corn meal to a considerable degree since the mixture of the two products induced a materially larger gain in weight per gram of protein consumed, both for the 30- and the 60-day tests, than did hog spleens alone. Likewise, the values of 3.51 g and 2.88 g obtained from the mixture for the 30- and 60-day tests, respectively, were materially higher than the calculated values of 2.65 g and 2.32 g.

Beef protein also supplemented that in corn meal materially since the mixture induced a larger gain in weight per gram of protein consumed than did beef alone. Furthermore, the actual gains in weight per gram of protein consumed in the mixture, both for the 30- and the 60-day tests (3.33 g and 3.07 g), were materially greater than the calculated values of 2.75 g and 2.44 g.

The experiments with cow udders and corn meal show that the udders alone induced a slightly greater gain in weight per gram of protein consumed during the 30-day test and a somewhat smaller gain during the 60-day test than the mixture of udders and corn meal. These results alone fail to disclose any supplementary relationship between the two kinds of protein in the 30-day test, and suggest only a slight supplementary value for the 60-day test. However, if the values determined through the experiment for the mixtures are compared with the calculated values, it appears probable that the two kinds of protein supplemented each other to a slight extent in the

[7] MITCHELL, H. H., and CARMAN, G. G. See footnote 5.

30- and the 60-day tests. For the 30-day test the calculated value was 2.45 g as compared with the determined value of 2.59 g, with a difference of +0.14 g, and for the 60-day test the values were 2.12 g and 2.41 g, respectively, with a difference of +0.29 g.

The experiment with a mixture containing 2 percent each of protein from hog spleens, calf lungs, cow udders, and cracklings failed to disclose any supplementary relationship among the several kinds of protein. The nutritive value of the mixed proteins was similar to that of cow udders alone.

The experiment with a mixture containing 1 percent each of protein from hog spleens, calf lungs, cow udders, and cracklings, and 4 percent of corn protein indicated a slight supplementary relationship between the animal and the corn proteins. The calculated gain in weight per gram of protein consumed in 30 days was 2.52 g, as compared with the experimental value of 2.64 g, with a difference of +0.12 g. The calculated value for the 60-day test was 2.20 g, as compared with the experimental value of 2.59 g, with a difference of +0.39 g.

SUMMARY

In this paper are reported the results of feeding experiments with young male albino rats to determine (1) the nutritive value, for maintenance and growth, of the protein in calf lungs, cow udders, and hog spleens as compared with that in beef; (2) the supplemental value of the proteins in the several animal products for one another; (3) the supplemental value of the protein in each animal product for that in corn meal; and (4) the supplemental value of a mixture of the proteins in several animal products for the protein in corn meal. The results of the experiments indicate the following:

(1) Beef had the highest value as a source of protein for maintenance and growth, followed closely by calf lungs and hog spleens, whereas cow udders had much the lowest value.

(2) The proteins in calf lungs, hog spleens, cow udders, and cracklings did not seem to supplement one another materially.

(3) The protein in calf lungs, hog spleens, and beef each supplemented the protein in corn meal to a considerable degree. The protein in cow udders appeared to supplement corn protein to a slight extent.

(4) The proteins in a mixture of hog spleens, calf lungs, cow udders, cracklings, and corn meal appeared to supplement one another to a slight degree.

EFFECT OF DIET, RANGE, AND FATTENING ON THE PHYSICAL AND CHEMICAL COMPOSITION OF COCKERELS [1]

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INTRODUCTION

A large proportion of the chickens sold for food in the United States are prepared for market in commercial feeding stations. The birds are confined in batteries and are fed heavily for various lengths of time on a wet mash which usually contains milk in some form. One major object of this procedure is to improve the quality of the carcasses of the birds.

The composition of the finished product of the poultry-fattening plants is one measure of the quality of the product, and may be affected by a number of factors. Of these the fattening process itself, carefully controlled in the feeding stations, is naturally of considerable importance. Other factors, not so easily controlled, are the diet of the birds previous to fattening and the degree of confinement during the rearing period. The latter is particularly important because of the increasing tendency of producers to rear their poultry in confinement. A study of the effect of all these factors on the composition of chickens should be of value, not only to those who fatten poultry for market, but to those who produce the birds as well.

PREVIOUS INVESTIGATIONS

Investigations of the effect of fattening on the chemical composition of poultry have been reported by Pfeiffer (*9*) [2] and Köhler (*5*). These investigations, however, were carried out more than 30 years ago, were made on a very limited number of birds, and did not involve an analysis of the various edible parts.

A few more recent contributions, which give the composition of chickens before and after fattening, are to be found in the literature, but the various conditions during the rearing period apparently have not been studied in this way. Lee (*6*) and Mairs (*7*) have reported the percentage which the dressed weight is of the live weight, the former investigator in fattened birds and the latter in unfattened birds. Jull and Maw (*4*) and Maw (*8*) have reported the percentage of dressed weight and of the edible portions in broilers and roasting chickens before and after fattening. Hepburn and Holder (*3*) have reported the results of physical and chemical analyses of fattened and unfattened birds.

[1] Received for publication Mar. 31, 1936; issued October 1936. The analyses reported in this paper were obtained as a part of a larger investigation known as the "McCollum feeding project." This project was carried on cooperatively by the U. S. Department of Agriculture, represented by the Bureau of Animal Industry, and members of the poultry industry, represented by E. V. McCollum, of the School of Hygiene and Public Health of Johns Hopkins University, and M. E. Pennington, consultant on the storage and refrigeration of foods.

[2] Reference is made by number (italic) to Literature Cited, p. 368.

The present paper reports a study of the effects of the diet fed during the rearing period, of range as compared with confinement, and of fattening on the physical and chemical composition of the edible portions of cockerels.

METHODS OF INVESTIGATION

Several groups of chickens were reared on various diets during 1927, 1928, and 1929. All of the birds were reared on range in 1927, but in 1928 and 1929 some of the birds on each diet were confined and some were allowed on range. An average of about 15 cockerels were reared in each group the first year, 32 the second year, and 60 the third.

Barred Plymouth Rock chicks were obtained from a commercial hatchery for the experiments of the first 2 years. During the third year's experiment, Rhode Island Red chicks, hatched from eggs produced by the breeding flock of the United States Animal Husbandry Experiment Farm, Beltsville, Md., were used.

In 1927, the chicks were hatched on April 18 and were put on feed 2 days later. The chicks raised during the second year were hatched on April 21 and were fed for the first time on April 24. In 1929, the chicks were hatched from two settings, one hatch occurring on April 8 and the other April 10, and were fed first on the day after hatching. All the chicks were hatched in incubators and brooded under similar conditions. They were kept in the brooder houses the first 2 days and allowed outdoors thereafter when the weather permitted. The range birds were brooded in colony brooder houses 10 feet square and were given access to a grass range 100 feet square. The confined birds were brooded in 8- by 15-foot rooms in a permanent brooder house and were allowed outdoors in 8- by 12-foot concrete-floored run yards.

The percentages of the ingredients used in the different diets are given in table 1. Separate groups of birds were reared on diets 0, 1, 2, 3, 4, 5, 7, and 27 in 1927; on diets 1, 2, 5, 6, 9, 10, and 29 in 1928; and on diets 1, 29, and 34 in 1929.

TABLE 1.—*Composition of the rearing diets used in experiments, 1927–29*

Diet no.	Corn, ground, yellow	Buttermilk, dried	Sodium chloride	Calcium carbonate	Bonemeal, steamed	Meat scrap	Meat meal, 72 percent protein	Fish meal	Pig liver, dried	Cod-liver oil	Menhaden fish oil	Alfalfa-leaf meal	Corn gluten meal	Bran, wheat	Middlings, wheat	Oats, rolled
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
0	40.0	4.0	1.0	2.0	3.0	....	8.0	.....	.....	.....	.....	2.0	.....	10.0	10.0	20.0
1	62.5	37.5	....	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2	62.2	37.3	....	....	....	....	....	.....	.....	0.5	.....	.....	.....	.....	.....	.....
3	61.9	37.1	....	....	....	....	....	.....	.....	.....	1.0	.....	.....	.....	.....	.....
4	60.6	36.4	1.0	2.0	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	60.3	36.2	1.0	2.0	....	....	....	.....	.....	.5	.....	.....	.....	.....	.....	.....
6	60.0	36.0	1.0	2.0	....	....	....	.....	1.0	.....	.....	.....	.....	.....	.....	.....
7	50.6	36.4	1.0	2.0	....	....	....	.....	.....	.....	.....	.....	.....	10.0	.....	.....
9	60.6	31.4	1.0	2.0	....	5.0	....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	60.6	31.4	1.0	2.0	....	....	....	5.0	.....	.....	.....	.....	.....	.....	.....	.....
27	50.6	36.4	1.0	2.0	....	....	....	.....	.....	.....	.....	10.0	.....	.....	.....	.....
29	60.6	26.4	1.0	2.0	....	....	....	.....	.....	.....	.....	.....	10.0	.....	.....	.....
34	50.6	16.4	1.0	2.0	....	....	....	.....	.....	.....	.....	.....	20.0	10.0	.....	.....

Diet 0 has given good results in rearing chickens at the experiment farm and is included for comparative purposes. Diet 1, the basal diet, was composed entirely of ground yellow corn and dried buttermilk mixed in such proportions as to contain approximately 18 percent of protein. The remaining diets contained various supplements to diet 1.

When the birds were nearly mature, the pullets were separated from the males and two representative cockerels from each group were killed and analyzed. At the same time, six other males from each group were placed in fattening batteries. The ages of these cockerels were 23 weeks in 1927, 25 weeks in 1928, and 20 weeks in 1929. The feed mixtures used for the fattening period in the respective years are shown in table 2. Twice each day the birds were allowed all they would consume of these wet mashes. After a 14-day fattening period, two representatives of the fattened birds from each group were killed and analyzed.

TABLE 2.—*Composition of the fattening mashes used in experiments, 1927–29*

Year	Corn, ground, yellow	Oats, rolled	Middlings, wheat	Flour, red-dog	Skim milk, dried	Buttermilk, dried	Water added
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1927	24	16			6		54
1928	24		3	12		7	54
1929	24	16				6	54

The birds were weighed just before they were killed. They were then bled, picked, and weighed again to obtain the dressed weights. Each of the carcasses was separated to obtain samples of breast muscle, leg muscle, and remaining edible portion. The last-mentioned sample was composed of all the edible material (including the heart, liver, and empty gizzard) except the breast and leg muscles. All the surface fat was removed from the breast and leg and was placed in the remaining edible sample, so that only that fatty tissue which was between or within the individual muscles of the breast and leg remained with these samples. The weights of the three samples were recorded.

The protein, fat, ash, and water of the breast muscle, the leg muscle, and the remaining edible portion were determined, and, from the results of these analyses, the composition of the total edible portion was calculated. The chemical determinations were made according to the methods of the Association of Official Agricultural Chemists (*1*): The water by distillation with toluene, the fat by extraction with ether, the ash by ignition to a red heat, and the nitrogen by the Kjeldahl method. The protein was calculated from the total nitrogen by use of the factor 6.25.

The results of the physical and chemical analyses of the individual cockerels were studied statistically by Fisher's (*2*) method of variance analysis.

RESULTS OF THE INVESTIGATION

PHYSICAL COMPOSITION

The results of the physical analyses of the birds are presented in tables 3, 4, and 5. The data on individual birds were too numerous to warrant their presentation in full, but the averages obtained under the different conditions each year and for the 3 years combined are presented in these tables.

TABLE 3.—*Ratios of the various edible portions of the cockerels, reared on the different diets, to their dressed weights*

Diet	Year	Birds	Breast muscle	Leg muscle	Remaining edible portion	Total edible portion
		Number	*Percent*	*Percent*	*Percent*	*Percent*
0	1927	4	13.1	21.1	21.8	56.0
1	1927	4	12.7	19.3	24.8	56.8
	1928	8	13.2	19.3	22.4	54.7
	1929	8	13.0	18.7	17.6	49.3
	Total or average	20	13.0	19.1	21.0	52.9
2	1927	4	12.3	19.8	20.6	52.7
	1928	8	13.5	19.7	22.0	55.3
	Total or average	12	13.1	19.8	21.6	54.4
3	1927	4	13.6	19.8	21.8	55.2
4	1927	4	12.3	19.9	23.8	56.0
5	1927	4	13.2	19.9	23.6	56.7
	1928	8	11.7	19.1	22.4	53.3
	Total or average	12	12.2	19.4	22.8	54.4
6	1928	8	13.3	19.7	21.9	54.9
7	1927	4	13.2	20.6	20.8	54.6
9	1928	8	12.1	19.6	23.0	54.7
10	1928	8	13.0	19.8	20.5	53.4
27	1927	4	12.0	19.7	22.9	54.6
29	1928	8	13.0	20.5	22.7	56.1
	1929	8	12.9	18.7	17.3	48.9
	Total or average	16	13.0	19.6	20.0	52.5
34	1929	8	12.9	19.4	18.0	50.4

TABLE 4.—*Ratios of the various edible portions of the cockerels, reared in confinement and on range, to their dressed weights*

Year	Birds	Breast muscle			Leg muscle			Remaining edible portion			Total edible portion		
		Confined birds [1]	Range birds	Difference [2]	Confined birds [1]	Range birds	Difference [2]	Confined birds [1]	Range birds	Difference [2]	Confined birds [1]	Range birds	Difference [2]
	No.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1927	32		12.80			20.01			22.52			55.34	
1928	28	12.36	13.32	7.8	18.73	20.63	10.1	21.45	22.83	6.4	52.55	56.69	7.9
1929	12	12.85	13.01	1.2	18.07	19.80	9.6	17.23	18.05	4.8	48.15	50.86	5.6
Total or average	72	12.51	13.04	4.2	18.53	20.22	9.1	20.18	21.90	11.1	51.23	55.12	7.6

[1] No birds were reared in confinement in 1927.
[2] Percentage increase in weight of birds reared on range.

TABLE 5.—*Ratios of the various edible portions of the cockerels, some before and others after fattening, to their dressed weights*

Year	Birds	Breast muscle			Leg muscle			Remaining edible portion			Total edible portion		
		Before fattening	After fattening	Difference[1]	Before fattening	After fattening	Difference[1]	Before fattening	After fattening	Difference[1]	Before fattening	After fattening	Difference[1]
	No.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1927	16	12.97	12.63	−2.6	20.12	19.89	−1.1	20.02	25.02	+25.0	53.15	57.53	+8.2
1928	28	13.14	12.70	−3.3	19.02	20.02	+5.3	21.24	23.24	+9.4	53.96	55.41	+2.7
1929	12	12.94	12.95	+1.1	18.48	19.50	+5.5	15.95	19.41	+21.7	47.36	51.81	+9.4
Total or average	56	13.05	12.73	−2.5	19.49	19.87	+1.9	19.75	22.97	+16.3	52.30	55.28	+5.7

[1] Percentage gain or loss during fattening period.

The results of the statistical analysis brought out the fact that on the whole the diets on which the birds were reared in this experiment had little effect on their physical composition. However, table 3 shows that the proportion of the breast muscle in the birds reared on diet 5 in 1928 was 10 percent less, on an average, than that of the birds reared on diets 1, 2, 6, 10, and 29. This difference was significant, the odds being greater than 19 to 1 that it was not due to chance.

The proportion of the edible portions in the carcasses of the birds reared on range was consistently higher than that of the birds reared in confinement. In the percentage which the breast muscle is of the dressed weight, the range birds were about 8 percent higher in 1928 than the confined birds. The proportion of leg muscle was about 10 percent greater in the range birds than in the confined birds in both 1928 and 1929. These differences were highly significant, the odds being more than 99 to 1 that they were not due to chance. Likewise, the proportion of total edible portion was 7.9 percent greater in 1928 and 5.6 percent greater in 1929 in the range birds than in the confined. The odds were greater than 99 to 1 in 1928 and 19 to 1 in 1929 that these differences in the total edible portions were not due to chance. The percentage of remaining edible portion was also higher in the range birds, but the difference was not consistent enough to be statistically significant.

Between the fattened and unfattened birds, little difference was found in the percentages of breast muscle and leg muscle and these differences were not significant. However, there was an increase in the weight of these edible portions during the 2-week fattening period. This increase in weight was 14 percent in the case of the breast muscle and 19 percent in that of the leg muscle, on an average, for the 3 years. The greatest and most consistent differences in the results of the physical analyses were found in the remaining edible portion of the carcasses analyzed before and after fattening. The carcasses of the fattened birds also contained a greater percentage of total edible portion than did those of the unfattened, the differences ranging from about 3 to 9 percent for the different years.

CHEMICAL COMPOSITION

The average chemical composition of the edible portions of the cockerels reared under the different conditions of diet, range, and fattening is shown in tables 6, 7, and 8.

TABLE 6.—*Average chemical composition of the edible portions of the cockerels reared on the different diets*

Diet no.	Year	Birds	Breast muscle				Leg muscle			
			Protein	Fat	Ash	Water	Protein	Fat	Ash	Water
		Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	1927	4	24.3	0.79	1.11	73.5	21.0	3.74	1.12	73.5
1	1927	4	24.3	.87	1.11	73.3	20.9	4.80	1.04	73.0
	1928	8	24.2	.91	1.11	74.3	20.6	4.70	1.08	73.9
	1929	8	24.5	.51	1.05	73.7	21.0	3.87	1.02	74.1
	Total or average	20	24.3	.74	1.09	73.9	20.8	4.39	1.05	73.8
2	1927	4	24.1	.65	1.14	73.9	20.8	4.86	1.04	73.1
	1928	8	24.3	.84	1.09	74.5	20.7	4.79	1.05	74.1
	Total or average	12	24.2	.78	1.11	74.3	20.7	4.81	1.05	73.7
3	1927	4	24.4	.88	1.22	73.6	20.8	4.98	1.09	72.9
4	1927	4	24.8	.82	1.14	73.3	21.3	4.48	1.12	73.0
5	1927	4	24.5	.65	1.24	73.5	21.1	4.42	1.05	72.8
	1928	8	23.8	.78	1.07	74.7	20.6	4.40	.99	74.1
	Total or average	12	24.0	.74	1.13	74.3	20.8	4.41	1.01	73.7
6	1928	8	24.3	.78	1.11	74.3	20.7	4.25	1.00	74.2
7	1927	4	24.6	1.03	1.14	73.7	21.1	4.70	1.12	73.0
9	1928	8	23.9	1.03	1.03	74.3	20.1	5.46	.97	73.6
10	1928	8	23.9	.84	1.05	74.3	20.1	4.58	1.02	74.6
27	1927	4	24.5	.98	1.13	73.6	20.7	4.93	1.09	73.3
29	1928	8	24.3	.99	1.06	74.1	20.7	4.47	.98	74.2
	1929	8	24.5	.45	1.07	73.8	21.3	3.73	1.02	74.0
	Total or average	16	24.4	.72	1.06	74.0	21.0	4.10	1.00	74.1
34	1929	8	24.6	.55	1.07	73.8	21.1	4.06	1.02	74.1

Diet no.	Year	Birds	Remaining edible portion				Total edible portion			
			Protein	Fat	Ash	Water	Protein	Fat	Ash	Water
		Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	1927	4	17.2	30.7	0.75	51.4	20.3	13.7	0.97	64.7
1	1927	4	15.6	36.4	.70	47.3	19.2	18.1	.90	61.6
	1928	8	17.8	25.1	.80	56.3	20.3	12.4	.97	66.6
	1929	8	19.2	21.9	.82	59.3	21.3	9.5	.96	68.2
	Total or average	20	17.9	26.1	.79	55.7	20.5	12.4	.95	66.2
2	1927	4	17.0	28.6	.77	53.7	20.0	13.2	.95	65.6
	1928	8	16.8	30.5	.69	52.2	20.0	14.1	.91	65.4
	Total or average	12	16.9	29.9	.72	52.7	20.0	13.8	.92	65.5
3	1927	4	16.3	32.0	.72	50.4	19.9	14.9	.97	64.0
4	1927	4	15.9	34.4	.71	48.4	19.7	16.7	.95	62.6
5	1927	4	15.9	34.7	.68	48.5	19.7	16.2	.95	62.9
	1928	8	17.5	26.8	.76	54.9	19.9	13.4	.91	63.9
	Total or average	12	17.0	29.4	.73	52.7	19.8	14.3	.92	63.5
6	1928	8	18.3	24.0	.80	57.2	20.6	11.4	.95	67.3
7	1927	4	17.5	27.8	.74	54.1	20.5	12.8	.98	65.9
9	1928	8	16.8	30.5	.76	51.8	19.5	15.3	.89	63.6
10	1928	8	18.6	22.2	.85	58.4	20.3	10.8	.96	68.0
27	1927	4	16.0	33.7	.67	49.7	19.4	16.4	.93	63.1
29	1928	8	17.5	29.0	.79	53.0	20.2	13.6	.92	65.6
	1929	8	19.7	20.8	.78	59.8	21.6	9.0	.95	68.8
	Total or average	16	18.6	24.9	.78	56.4	20.9	11.3	.93	67.2
34	1929	8	19.0	22.6	.83	58.4	21.2	9.9	.96	68.3

TABLE 7.—*Average chemical composition of the edible portions of the cockerels reared in confinement and on range*

BREAST MUSCLE

Year	Birds	Protein content of—		Fat content of—		Ash content of—		Water content of—	
		Confined [1] birds	Range birds	Confined [1] birds	Range birds	Confined [1] birds	Range birds	Confined [1] birds	Range birds
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1927	32	.....	24.4	.....	0.83	.....	1.15	.....	73.5
1928	28	23.7	24.4	0.85	.92	1.06	1.08	74.6	74.1
1929	12	24.6	24.5	.47	.53	1.07	1.07	73.6	74.0
Total or average	72	24.0	24.4	.74	.81	1.06	1.11	74.3	73.8

LEG MUSCLE

1927	32	.....	21.0	.....	4.55	.....	1.08	.....	73.1
1928	28	20.1	20.9	4.96	4.36	0.99	1.04	74.2	74.0
1929	12	21.2	21.1	3.62	4.15	1.02	1.02	74.3	73.9
Total or average	72	20.4	21.0	4.56	4.41	1.00	1.05	74.2	73.6

REMAINING EDIBLE PORTION

1927	32	.....	16.4	.....	32.3	.....	0.72	.....	50.5
1928	28	17.8	17.4	24.9	28.9	0.80	.76	56.6	53.0
1929	12	19.5	19.1	21.1	22.4	.82	.80	59.9	58.4
Total or average	72	18.3	17.3	23.8	29.3	.81	.75	57.6	52.8

TOTAL EDIBLE PORTION

1927	32	.....	19.8	.....	15.3	.....	0.95	.....	63.8
1928	28	19.9	20.3	12.4	13.6	0.93	.93	66.9	65.4
1929	12	21.4	21.2	9.1	9.8	.97	.95	68.7	68.0
Total or average	72	20.4	20.2	11.4	13.7	.94	.94	67.5	65.1

[1] No birds were raised in confinement in 1927.

There were practically no differences in the chemical composition of the edible portions of the cockerels which could be ascribed to diet. In three instances, once in breast muscle and twice in leg muscle, there was a difference of about 5 percent in the proportion of ash which could be attributed to diet, but the constituent of the diet responsible for this difference could not be determined.

The results of the chemical analyses reveal a few significant differences between the composition of the range birds and the confined birds. In 1928, in the leg muscle of the range birds the percentage of protein was almost 4 percent higher and that of the ash was more than 5 percent higher than in the confined birds. In the same year, the percentage of protein in the breast muscle was almost 3 percent greater in the range birds. These differences were all highly significant. The percentage of fat in the edible portions was generally higher in the range birds, but these differences were not statistically significant.

TABLE 8.—*Average chemical composition of the edible portions of the cockerels, some before and others after fattening*

BREAST MUSCLE

Year	Birds	Protein content—		Fat content—		Ash content—		Water content—	
		Before fattening	After fattening	Before fattening	After fattening	Before fattening	After fattening	Before fattening	After fattening
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1927	16	24.4	24.5	0.68	0.98	1.16	1.14	74.0	73.1
1928	28	24.1	24.0	.58	1.19	1.10	1.05	74.6	74.1
1929	12	25.1	23.9	.33	.67	1.13	1.00	73.6	74.0
Total or average	56	24.4	24.1	.55	1.02	1.12	1.07	74.2	73.8

LEG MUSCLE

1927	16	21.4	20.6	3.62	5.48	1.10	1.07	73.3	72.9
1928	28	20.8	20.2	4.04	5.29	1.04	.99	74.4	73.8
1929	12	21.5	20.8	2.92	4.85	1.07	.97	74.5	73.7
Total or average	56	21.1	20.4	3.68	5.25	1.06	1.01	74.1	73.5

REMAINING EDIBLE PORTION

1927	16	18.3	14.6	24.8	39.8	0.80	0.64	56.1	44.9
1928	28	19.0	16.2	21.8	32.0	.84	.71	58.5	51.2
1929	12	21.2	17.4	15.7	27.8	.89	.73	63.6	54.6
Total or average	56	19.3	16.0	21.3	33.3	.84	.70	58.9	50.1

TOTAL EDIBLE PORTION

1927	16	20.9	18.8	10.9	19.6	1.00	0.90	66.9	60.7
1928	28	20.8	19.4	10.4	15.7	.97	.89	68.0	64.3
1929	12	22.4	20.3	6.5	12.4	1.03	.89	70.6	66.3
Total or average	56	21.2	19.4	9.7	16.1	.99	.89	68.3	63.7

Table 8 shows marked and consistent differences in composition between the fattened and unfattened birds. The percentage of fat was higher in all the edible portions of the fattened birds than it was in the same portions of the unfattened ones. The differences were about 85 percent in the breast muscle, 43 percent in the leg muscle, 57 percent in the remaining edible portion, and 66 percent in the total edible portion. However, in the breast muscle there was only 0.55 percent of fat on an average, before fattening, and the actual gain in the weight of the breast fat was, therefore, very small.

There was usually a lower percentage of protein, ash, and water in the edible portions of the fattened birds. These differences were uniformly significant in the remaining and total edible portions and, in most cases, in the breast and leg muscles. Attention is called to the fact that there was a gain in the weight of the protein, ash, and water, even though there was a decrease in the percentage of these constituents.

COMPOSITION OF THE GAIN IN WEIGHT DURING FATTENING

By means of the results obtained from the physical and chemical analyses of these cockerels, the composition of the gain in weight during the fattening period was calculated. A summary of the increase in the live weight, dressed weight, and in weight of the edible portions is given in table 9. The increase in the chemical constituents of the edible portions is presented in table 10.

TABLE 9.—*Average weights of the cockerels, some before and others after fattening, and of the edible portions of their carcasses, the gain in weight during fattening, and the relation of these weights and gains to the total live weight before fattening*

[Averages of 56 fattened and 56 unfattened cockerels]

Item	Before fattening		After fattening		Gain during fattening	
	Weight	Live weight before fattening	Weight	Live weight before fattening	Weight	Total live weight before fattening
	Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
Live weight	2,203	100.00	2,530	114.84	327	14.84
Dressed weight [1]	1,878	85.25	2,196	99.68	318	14.43
Breast muscle	245	11.12	280	12.71	35	1.59
Leg muscle	366	16.61	437	19.84	71	3.23
Remaining edible	371	16.84	505	22.92	134	6.08
Total edible	982	44.57	1,222	55.47	240	10.90

[1] The dressed weight is the weight of the carcass after killing and picking.

TABLE 10.—*Chemical distribution of the gain in weight of the edible portions of the cockerels during fattening*

[Average difference between 56 fattened and 56 unfattened cockerels]

Edible portion	Protein		Fat		Ash		Water		Total gain	
	Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
Breast muscle	8	3.2	1.5	0.6	0.25	0.1	25	10.4	34.75	14.3
Leg muscle	12	5.0	9.0	3.7	.53	.2	50	20.8	71.53	29.7
Remaining edible portion	10	4.2	89.0	37.1	.42	.2	35	14.5	134.42	56.0
Total edible portion	30	12.4	99.5	41.4	1.20	.5	110	45.7	240.70	100.0

The increase in the total edible portions was about 73 percent, that of the remaining edible portion 41 percent, that of the leg muscle 21 percent, and that of the breast muscle 10 percent of the gain in live weight during fattening. In the edible portion alone, the gain in protein was 9 percent; in fat, 30 percent; in ash, about 0.4 percent; and in water, 33 percent of the gain in live weight.

These results show that the gain in weight of the birds consisted largely of an increase in fat and water in the parts included in the remaining edible portion, chiefly in fat deposits about the abdominal organs and under the skin. At the same time there was a noticeable gain in the other constituents and in the breast and leg muscle.

DISCUSSION

The fact that the birds reared on range had a higher percentage of breast muscle, leg muscle, and total edible portion than those reared in confinement is probably due, in part, to the greater opportunity for exercise afforded the range birds and, in part, to the feed which the birds were able to pick up on the range. Any factor which results in a higher proportion of breast or leg muscle merits practical consideration, for these are the most desirable portions from the consumer's standpoint, and they play a large part in determining the market grade of the finished carcass.

The results of this investigation have shown that, during the fattening period, relatively large quantities of fat and water were deposited in the adipose tissues of the bodies of the cockerels. At the same time there was an increase in the weight of water and fat in the breast and leg muscle and a small gain in the weight of protein and ash in all the edible tissues.

Since the birds used in these experiments were not mature when they were fattened, they would be expected to continue their growth during the fattening period. This continued growth accounts for the increase in the quantity of ash and protein and for at least part of the water. Such a gain is probably no greater than would have occurred on ordinary feeding for the same length of time. In young, rapidly growing birds, the increase in protein and ash would make up a greater proportion of the gains than in mature birds.

It has been frequently stated in the literature that a part of the water in the tissues of chickens is replaced by fat during the fattening process. Such a statement is probably based on the fact that the percentage of water in the tissues decreases with an increase in the percentage of fat. This decrease in the proportion of water, as well as that of the ash and protein, is a result of the diluting effect of the large deposition of fat in the tissues. As a matter of fact, there was an increase in the quantity of water, ash, and protein in all the edible portions. In the case of water, this increase in quantity was 10 percent greater than that of the fat.

Perhaps the picture may be presented more clearly if a calculation of the ratio of water to nitrogen in the edible portions is given. The results of this computation are presented in table 11, which shows that the ratio of water to nitrogen was slightly higher in every case after fattening than before and, in the gain during fattening, it was higher than either before or after fattening. Thus the proportion of water in the fat-free edible tissues tends to increase during fattening.

TABLE 11.—*Parts of water to 1 part of nitrogen in the edible portions of the cockerels, some before and others after fattening, and in the tissues constituting gain in weight during fattening*

Edible portion	Parts of water to 1 part of nitrogen in—		
	Edible portions before fattening	Edible portions after fattening	Gain during fattening
Breast muscle	19.01	19.11	19.50
Leg muscle	21.96	22.47	26.07
Remaining edible portion	19.06	19.63	21.85
Total edible portion	20.14	20.54	23.48

Table 11 also shows that the leg muscle contains a considerably greater proportion of water than the other edible portions. This difference was consistent in the fattened birds, in the unfattened birds, and in the composition of the gain during fattening.

SUMMARY

Several groups of chickens were reared each year for 3 years on a number of different diets, and, during the last 2 years, under opposite conditions of range and confinement. The diets fed were as follows: A normal diet which had given good results in rearing chickens and which was included for comparative purposes, a basal diet composed entirely of ground yellow corn and dried buttermilk, and a number of other diets which were formulated by substituting mineral supplements, fish oils, animal byproducts, or plant products in the basal diet.

When the birds were nearly mature, representative cockerels were selected from each group and analyzed. Other cockerels from each group were fattened for 2 weeks, and analyses were again made of representative birds.

For the purpose of making physical and chemical analyses, the edible portions of the carcasses were separated into 3 parts—the breast muscle, the leg muscle, and the remaining edible portion—and these parts were weighed. The percentages of protein, fat, ash, and water were then determined in each of these portions, and from the results the percentage composition of the total edible portion was calculated. The average results of these analyses are presented. The results were studied statistically by Fisher's method of variance analysis.

The study showed that the rearing diets used did not result in any significant differences in the physical composition of the birds in a great majority of cases.

The percentage of breast muscle was about 8 percent higher in 1928, that of leg muscle was about 10 percent higher in 1928 and 1929, and that of the total edible portion was about 8 percent higher in 1928 and 6 percent in 1929 in those birds which were reared on range than in those which were confined.

Fattening resulted in increases in the proportion of remaining edible portion (comprising all the edible portions except the breast and leg muscle), which ranged from nearly 10 percent in 1928 to 25 percent in 1927. In total edible portion there was an average increase of almost 6 percent for the 3 years due to fattening. No significant change in the percentage of breast or leg muscle took place during fattening, though there were gains of 14 percent in the weight of the breast muscle and 19 percent in that of the leg muscle.

The diets used had relatively little effect on the chemical composition of the edible portions. A few significant differences were found in the ash content of the breast and leg muscles of the birds reared on some of the diets, but the constituent responsible for these differences could not be determined.

In only 1 of the 2 years in which range was compared with confinement was there any significant chemical difference due to this factor. In 1928, the proportion of protein in the breast muscle was almost 3 percent greater, that of protein in the leg muscle was almost 4 per

cent greater, and that of ash in the leg muscle was 5 percent greater in the range birds than in the confined.

The birds which were fattened for 2 weeks had, on an average, 85 percent more fat in the breast muscle, 43 percent in the leg muscle, 57 percent in the remaining edible portion, and 66 percent in the total edible portion, than those which were not fattened.

The gain in live weight of the birds during fattening, as calculated from the analytical results, consisted of about 73 percent in the total edible portions, 41 percent in the remaining edible portions, 21 percent in the leg muscle, and 10 percent in the breast muscle. The gain in protein was 9 percent, that of the fat 30 percent, that of the ash 0.4 percent, and that of the water, 33 percent of the gain in live weight.

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THE RELATIVE VALUE OF VARIOUS LARDS AND OTHER FATS FOR THE DEEP-FAT FRYING OF POTATO CHIPS [1]

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INTRODUCTION

Notwithstanding the increase in the large-scale preparation of potato chips and other foods fried in deep fat there has been little scientific investigation published on the relative value of the various fats used for this purpose. To meet the demand for such information the Bureaus of Home Economics, Animal Industry, and Plant Industry during 1933 undertook cooperative experiments on nine fats used in the preparation of potato chips. Throughout these studies it was considered that a satisfactory fat for frying should be stable; should have a high smoking point and not deteriorate unduly during frying; and should produce a high-quality product that can be merchandised without too great a loss of quality.

REVIEW OF LITERATURE

A number of the studies made to determine the effects of various conditions of cookery upon fats have a bearing on this question of fats for deep frying. An early investigation by Fulmer and Manchester (*5*) [3] showed that the decrease in iodine value for cottonseed oil was hastened when the oil was heated above 180° C., but that temperatures below 220° produced little change in the free fatty acid content. Between 220° and 240° the acid percentage was more than doubled when the oil was heated for 10 minutes; after 30 minutes it was 4 times as great as in the original oil; and at 270° it was 9 and 15 times as great when the oil was heated for 10 and 30 minutes, respectively.

Upon comparing cottonseed oil and butter when fresh and when extracted from pastry baked at 200° C., Masters and Smith (*9*) found a decrease in iodine values and an increase in acetyl value, refractive index, and acidity in the heated fats. They concluded that the first change in heated fats might be hydroxylation of the unsaturated molecule. The oxidation was apparently measurable only when the fat was overcooked.

Blunt and Feeney (*2*) found that fat which has been used in cooking had both a lower smoking point and a higher acidity than did fresh fat.

Holmes and Lang (*6*) concluded that doughnuts fried in beef fat, lard, cottonseed oil, coconut fat, and peanut oil had the least fat

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[3] Reference is made by number (italic) to Literature Cited, p. 381.

absorption if the vegetable fats were heated 30° to 40° F. higher than the animal fats.

Cooking experiments conducted by Williams and Gray (*16*) led them to conclude that fat absorption was dependent more upon the kind of food and the temperature used than upon the kind of frying fat. They advocated the use of stable fats having little flavor, such as cottonseed products.

When Woodruff and Blunt (*17*) fried potato chips and doughs in lard and cottonseed oil they found that a longer period was required to secure the desired brown color of the product when lard was used. Potato chips fried in lard at 190° C. gave a fat absorption of 34.18 percent, while chips fried in cottonseed oil absorbed 34.4 percent. With doughnuts the amount of fat absorbed depended upon the temperature used and the period of frying, but not so with potato chips. The fat extracted from the foods fried in cottonseed oil showed less change in iodine number and acidity value than did that fried in lard. Woodruff and Blunt believed that the initial acidity of the lard accounted in part for its high acidity after heating. They found so little change in the oil that the effect of the time of frying was considered to be negligible. On the other hand, the fat absorbed by the fried food underwent greater chemical changes than the frying fat. They concluded that the appearance of a lard after frying gives no indication of its quality.

Morgan and Cozens (*10*) found that the fats used in frying doughs showed consistent decreases in iodine number and melting point but increases in acidity and refractive index after frying. They thought it possible that a relationship existed between the acidity of the fat and the quantity of fat absorbed by dough fried in lard, olive oil, and hydrogenated cottonseed oil at 210° C.

Sprague (*13*) stated that the reheating of a fat seemed to have little effect upon its quality if the temperature of the fat was kept low.

Vosbury (*15*), using leaf lard, a mixture of lard and suet, hydrogenated cottonseed oil, and cottonseed, coconut, and peanut oils, reported that a high-grade cottonseed oil was the most satisfactory fat for frying potato chips.

Porter, Michaelis, and Shay (*11*) concluded after frying doughs in animal fat, vegetable oil, and three hydrogenated vegetable fats, that the breaking down of fat involving the formation of acid was a direct result of a reaction with water at elevated temperatures. Up to approximately 0.75 percent acid the rate was considerably greater. Doughs fried in a hydrogenated vegetable fat between the temperatures of 176.5° to 199° C. had an objectionable flavor when about 2 percent of acid (as oleic) was present.

Coe (*3*) through extensive investigations has shown that oil-bearing foods subject to rancidity may be protected from deleterious wave lengths of light by enclosing them in green containers or wrappers of a proper shade. He has given a comprehensive review of the literature in relation to the rancidity problem.

MATERIALS AND METHODS

The following fats were used for the cooking tests:

Three kettle-rendered lards produced by the Bureau of Animal Industry at the National Agricultural Research Center of the United

States Department of Agriculture, Beltsville, Md., from animals fed on rations consisting largely of (1) peanuts, (2) corn, and (3) brewer's rice. These lards were prepared according to the tentative method adopted by the 1932 Conference on Cooperative Meat Investigations. They are referred to in this paper as "peanut", "corn", and "rice" lards.

The peanut, corn, and brewer's rice rations were used to give characteristically soft or oily, medium hard to hard, and very hard lards, respectively. Six representative commercial fats were used, namely, a standard prime steam lard (an unrefined product of the wholesale trade), a hydrogenated lard, a hydrogenated cottonseed oil, a highly refined corn oil, a highly refined cottonseed oil, and a highly refined peanut oil. The commercial fat samples were obtained directly from the manufacturers at about the time the lards were rendered at Beltsville.

All the fats were stored in 5-pound covered tin containers and kept at a temperature of from 4.4° to 7.5° C. throughout the experiment.

The potatoes were of the Green Mountain variety and were harvested from the Bureau of Plant Industry plots at Presque Isle, Maine, by October 1. They were held in storage at a temperature of 16° C. during the period of the experiment, since Sweetman (*14*) and Wright and his associates (*18*) had reported that potatoes stored at a lower temperature develop too much sugar to make a desirable potato chip.

The potatoes were washed and allowed to dry overnight. The blemishes were removed from a weighed quantity, then the unpared potatoes were sliced 1.5 mm in thickness by means of a rotary hand slicer. Four kilograms of potato slices were needed for the whole series of fryings made in 1 day. Approximately 500 g were sliced at one time, washed for 2 minutes, then dried thoroughly between towels and placed in tightly covered jars. When the entire 4 kg had been so treated, the slices were all mixed and returned to the jars.

For frying, 2 kg of each fat was heated to 185° C. in a 5-quart iron kettle 7.75 inches in diameter. The temperature was held stationary for one-half minute and then a wire basket containing 100 g of potato slices was lowered into the fat. The slices were stirred with a wire fork during the 2-minute frying period. The frying operation was continued in each fat until the 400-g sample of potato slices was fried. After the four fryings, each fat was strained through cheesecloth and placed in a covered pail in the refrigerator. All the fats were used for frying during 1 day, and the fryings were continued until 10 fryings had been made in each fat. As the quantity of each fat diminished a corresponding decrease was made in the quantity of potato slices used, so that the weight ratio of fat to slices was kept at 5 to 1. The potato chips were drained on absorbent paper, weighed, and stored in tightly covered jars. At the end of the tenth frying the experiment was repeated and fresh fat was used. The two sets of fryings are referred to as series 1 and series 2.

A record was kept of the temperature readings for each fat at the end of each half minute during the frying of the chips.

To make the moisture and fat determinations, samples of chips from each fat were ground in a mortar immediately after frying, and dried to constant weight at 100° C. The loss was calculated as mois-

ture. The dried material was extracted for 18 hours with anhydrous ether in a Soxhlet extraction apparatus and the fat content calculated. These determinations were repeated on the chips from the first and the tenth fryings.

The day after frying, the potato chips were judged by a committee of three persons representing the Bureaus of Home Economics and Plant Industry. The record card (fig. 1) included color, luster, aroma, crispness, oiliness, and flavor. Each judge also gave a general opinion in terms of very poor, poor, fair, good, and very good for all the chips included in the experiments.

Chemical and physical determinations, including those for peroxide value, iodine number, and free fatty acid (as oleic) were made on the

Record card for deep-fat frying

No. of Frying_____ Sample No._____ Product, Potato Chips

	Factor	3	2	1	Remarks
Color	Desirability	Desirable	Neutral	Undesirable	
Luster	Intensity	Bright	Moderately bright	Dull	
Aroma	Intensity	Slightly pronounced	Moderately pronounced	Very pronounced	
	Desirability	Desirable	Neutral	Undesirable	
Crispness	Intensity	Crisp	Moderately crisp	Slightly crisp	
Oiliness	Intensity	No oiliness	Moderately oily	Oily	
Flavor	Intensity	Slightly pronounced	Moderately pronounced	Pronounced	
	Desirability	Pleasant	Neutral	Unpleasant	

General Desirability: Very poor, poor, fair, good, very good.

_____
Signature of judge

FIGURE 1.—Reproduction of record card used for recording the general quality of potato chips.

fats, both before and during the frying operations, to determine the extent of deterioration. In addition, free fatty acid was determined in the fats extracted from chips in the first and tenth fryings with each of the fats. The extraction was carried out under essentially identical conditions of time and temperature, ether being used as the solvent.

Thirty-gram samples of chips from the first frying in each fat were stored in white glassine bags at room temperature (22.6° C.) and in the refrigerator (3.2 C.), in each case in the absence of light. Organoleptic tests for rancidity were made by the judges at weekly intervals until rancidity developed in all chips stored at room temperature.

EXPERIMENTAL DATA

FRYING TEMPERATURE READINGS

Preliminary frying tests with the potatoes that were slightly high in sugar showed that the most desirable chips were produced by frying for 2 minutes at an initial temperature of 185° C. The average

temperature readings for the two series taken at half-minute intervals during the 2-minute frying period of the chips were: 0.5 minute, 161.1°; 1 minute, 152.2°; 1.5 minutes, 149.3°; 2 minutes, 148.5°.

MOISTURE AND FAT DETERMINATIONS

Analysis of the raw potatoes showed 22.8 percent dry matter and 0.038 percent fat. Analysis of the potato chips after the first and tenth fryings indicated an average range of 1.4 to 1.9 percent moisture for the chips fried in all the fats. The fat absorption by the chips was about the same for all kinds of fat, the average for the first frying of series 1 and 2 being 42.07 percent and for the tenth frying of the two series 43.6 percent. The maximum and the minimum fat absorption in the first frying of series 1 and 2 was 42.93 percent and 40.26 percent, respectively. The maximum and the minimum fat absorption in the tenth frying of series 1 and 2 was 45.05 percent and 42.81 percent, respectively.

PROGRESSIVE DETERIORATION OF FATS

Chemical Changes

Determinations made on the fats (table 1) showed them to be reasonably fresh when first received. The lards, exclusive of the hydrogenated, showed higher values for free fatty acids than did the hydrogenated and refined oils. Analyses of the fats of both series after use showed increases in free fatty acid and in peroxide values, and decreases in iodine number. Generally speaking, these values indicated some deterioration through continued use of the fat.

Table 1.—*Free fatty acid, iodine number, and peroxide value of fats when unused and after use in 5 and 10 fryings of potato chips*

Kind of fat	Free fatty acid (as oleic acid)					Iodine number			Peroxide value		
	Unused fat	Series 1		Series 2		Unused fat	Series 1	Series 2	Unused fat	Series 1	Series 2
		Fifth frying	Tenth frying	Fifth frying	Tenth frying		Tenth frying	Tenth frying		Tenth frying	Tenth frying
	Percent	*Percent*	*Percent*	*Percent*	*Percent*				*Millimols O_2 per kg*	*Millimols O_2 per kg*	*Millimols O_2 per kg*
"Rice" lard	0.43	0.70	0.88	0.54	0.68	54.1	50.4	51.6	1.5	7.3	12.8
"Corn" lard	.38	.51	.56	.54	.60	59.4	54.1	54.9	1.3	14.7	21.1
"Peanut" lard	.20	.29	.38	.28	.41	91.5	86.8	86.6	2.9	19.0	24.9
Prime steam lard	.40	.50	.58	.45	.54	65.2	59.6	60.4	.3	11.6	16.0
Hydrogenated lard	.03	.13	.24	.10	.19	62.0	56.5	57.8	1.0	14.3	12.3
Hydrogenated cottonseed oil	.04	.11	.18	.08	.17	69.6	64.2	64.9	.4	12.3	15.6
Cottonseed oil	.03	.08	.15	.06	.10	.....	109.0	107.1	3.9	11.6	11.9
Corn oil	.05	.06	.14	.07	.14	128.2	125.1	121.8	4.6	10.1	11.3
Peanut oil	.13	.16	.26	.16	.23	.....	91.4	92.9	2.0	15.6	25.4

The free fatty acid values of the unused fats varied with the source of the fats and the method used in their manufacture. After use in 10 fryings of potato chips the increase in free fatty acid was less in the oils and in the hydrogenated cottonseed oil than in the lards. Decrease in iodine number amounted to as much as 5.6 units (prime steam) among the lards in series 1 and to 6.4 units for the corn oil in

series 2. The increases in peroxide values showed no outstanding tendency for any fat or group of fats.

While the determinations made at the end of the fifth frying (table 1) showed increases in fatty acid over values found in the unused fats, there was no indication that they could be considered as midpoints for the determinations made after the tenth fryings.

Color of the Fats

A comparison of the color of the different fats before the first and after the tenth frying showed greatest discoloration in the lards, with the exception of the hydrogenated lard. The least deterioration as indicated by color was in the oils. The change was slight and uniform for all the oils.

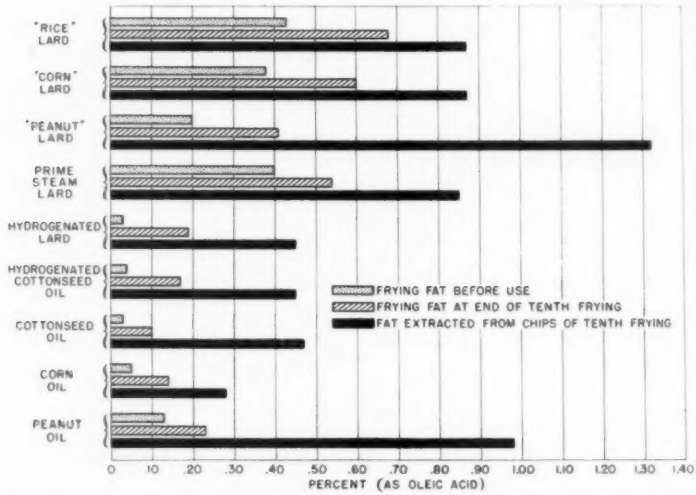
"RICE" LARD
"CORN" LARD
"PEANUT" LARD
PRIME STEAM LARD
HYDROGENATED LARD
HYDROGENATED COTTONSEED OIL
COTTONSEED OIL
CORN OIL
PEANUT OIL
FRYING FAT BEFORE USE
FRYING FAT AT END OF TENTH FRYING
FAT EXTRACTED FROM CHIPS OF TENTH FRYING
0 .10 .20 .30 .40 .50 .60 .70 .80 .90 1.00 1.10 1.20 1.30 1.40
PERCENT (AS OLEIC ACID)

FIGURE 2.—Changes in free fatty acid content of nine fats used as the cooking medium for potato chips.

The Fat in the Chips

As indicated by figure 2, the free fatty acid content of the fat extracted from the chips was higher in every instance than the fat in which the chips were fried.

Palatability of the Chips

The palatability values given in table 2 for each series are means derived from 30 independent judgments, that is, 3 judgments on the ships from each of 10 fryings.

In order to evaluate the judgments the ranking given to the chips fried in the different fats was averaged for each quality factor (table 2). By using Fisher's method (*4*) of analysis of variance to determine the significance of difference between the means it was found that differences were significant in the intensity of luster, desirability of flavor, and the general desirability arrived at, in the judging of the chips fried in the different fats. Snedecor's method (*12*) was followed for the other items on the grading chart.

TABLE 2.—*Average of the mean grades of the palatability factors used in judging potato chips fried in 9 fats during 10 fryings*

Palatability factor on which chips were judged	"Rice" lard	"Corn" lard	"Peanut" lard	Prime steam lard	Hydrogenated lard	Hydrogenated cottonseed oil	Cottonseed oil	Corn oil	Peanut oil
Color, desirability:									
Series 1	1.9	2.0	2.3	2.5	2.3	2.3	2.5	2.3	2.6
Series 2	2.2	2.4	2.7	2.6	2.3	2.5	2.7	2.7	2.9
Average	2.08	2.21	2.51	2.58	2.33	2.41	2.58	2.50	2.73
Luster, intensity:									
Series 1	1.2	1.2	2.3	1.5	1.4	1.6	2.4	2.2	2.4
Series 2	1.1	1.2	2.2	1.7	1.1	1.3	2.2	2.2	2.2
Average	1.13	1.19	2.21	1.56	1.23	1.46	2.33	2.21	2.29
Aroma, intensity:									
Series 1	1.9	1.9	1.6	2.0	2.3	2.3	2.3	2.2	2.4
Series 2	1.8	1.7	1.9	1.6	2.0	2.3	2.3	2.1	2.3
Average	1.86	1.84	1.75	1.78	2.14	2.29	2.29	2.17	2.36
Aroma, desirability:									
Series 1	1.6	1.6	1.3	1.8	2.0	2.2	2.2	2.1	2.3
Series 2	1.6	1.5	1.6	1.4	1.7	2.0	2.1	2.0	2.2
Average	1.53	1.55	1.46	1.60	1.83	2.13	2.15	2.10	2.25
Crispness, intensity:									
Series 1	3.0	2.9	3.0	2.9	3.0	3.0	3.0	2.9	2.9
Series 2	3.0	3.0	3.0	2.9	3.0	3.0	3.6	3.0	3.0
Average	2.96	2.93	2.98	2.93	2.98	2.96	2.98	2.93	2.96
Oiliness, intensity:									
Series 1	3.0	2.9	2.4	2.8	3.0	3.0	2.6	2.5	2.4
Series 2	2.9	3.0	2.3	2.7	3.0	2.9	2.3	2.4	2.3
Average	2.95	2.91	2.36	2.76	2.98	2.95	2.44	2.43	2.36
Flavor, intensity:									
Series 1	1.6	1.6	1.9	1.6	1.9	2.3	2.2	2.2	2.2
Series 2	1.7	1.6	2.3	1.2	1.9	2.1	2.2	2.3	2.4
Average	1.61	1.56	2.08	1.41	1.88	2.20	2.23	2.23	2.30
Flavor, desirability:									
Series 1	1.4	1.4	1.7	1.5	1.7	2.3	2.1	2.3	2.4
Series 2	1.4	1.5	1.9	1.1	1.5	2.0	2.1	2.3	2.5
Average	1.43	1.50	1.78	1.31	1.61	2.11	2.11	2.30	2.46
General desirability:									
Series 1	2.3	2.2	2.7	2.4	2.8	3.5	3.7	3.4	3.8
Series 2	2.2	2.4	3.0	1.9	2.6	3.1	3.4	3.5	3.9
Average	2.25	2.28	2.85	2.13	2.68	3.35	3.55	3.45	3.88

Tests of significance of the differences between the means were made for series 1 and 2 separately and combined. The results of the two series were remarkably corroborative. Where any difference occurred in the relative positions of two fats in the two series, it was found that on the whole the difference in means between them was insignificant or unimportant. The factors graded by the judges were considered in the order given on the grading chart.

Peanut oil, cottonseed oil, prime steam lard, peanut lard, and corn oil as a group were found to impart the most desirable color to the potato chips. Hydrogenated cottonseed oil and hydrogenated lard formed an intermediate group; and corn and rice lard a lower group which gave the least desirable color in the chips.

In the average of the two series of tests, cottonseed oil imparted the brightest luster to the chips, but the differences among cottonseed, peanut, and corn oil, and peanut lard were unimportant. These four formed one group; prime steam lard and hydrogenated cottonseed oil, an intermediate group; and the other lards the lowest group, which gave less luster to the chips.

In determining intensity of aroma the chips with the most pronounced aroma were considered of lowest grade. In both series of tests, peanut oil appeared to give the least pronounced aroma to the chips, but the differences between its effect and the effect of the other oils were not significant. Hydrogenated lard stood midway, more pronounced than the oils but less pronounced than the other lards. The differences among the group consisting of rice, corn, prime steam, and peanut lard, which gave the most pronounced aroma to the chips, were insignificant.

Peanut oil in both series seemed to impart to the chips the most desirable aroma, yet statistically it shows no significant difference from the other oils. The four oils formed one group, giving the most desirable aroma to the chip, while hydrogenated lard was intermediate, being less desirable than the oils but more desirable than the other lards. Prime steam, rice, corn, and peanut lard formed a homogeneous group neutral to undesirable in effect on aroma.

A close relationship existed between intensity and desirability of aroma. The aroma considered most desirable was the least pronounced. A close positive association also existed between the desirability of flavor and the desirability of aroma imparted to the chips by the fats. The only exception was peanut lard, which imparted a pronounced and undesirable aroma, but the flavor given bordered on the neutral and was not so undesirable as that of the other lards.

There was no significant effect in the crispness of the chips according to kind of fat used for frying. An examination of the averages showed that all the potato chips had practically the same crispness.

There was little difference between the chips as to oiliness. Hydrogenated lard, hydrogenated cottonseed oil, rice lard, and corn lard as a group produced the least oily chips. Chips fried in prime steam lard were significantly more oily than those listed above, but less oily than chips fried in cottonseed, corn, and peanut oil, and peanut lard. There were no significant differences among the last four.

In regard to intensity of flavor, the chips fell into three groups. The oils imparted a less pronounced flavor than the other two groups but were not significantly different from each other. Peanut lard and hydrogenated lard gave a more pronounced flavor than the oils but less pronounced than the other lards; and rice, corn, and prime-steam lard, gave the most pronounced flavor but were not significantly different from each other.

In both series of tests peanut oil was judged to impart the most desirable flavor to the chips. Corn oil came second to peanut oil in both series, and the two cottonseed oils third and fourth. Peanut lard and hydrogenated lard gave a less desirable flavor to the chips than the oils but a more desirable flavor than corn, rice, or prime-steam lard. The last three gave the least desirable flavor but were not significantly different from each other in their effect.

The judges placed the chips fried in the different fats, in respect to general desirability, in five grades: very poor, poor, fair, good, and very good.

Desirability of flavor was evidently the most important item in determining the general grade. For the two series combined, the fats occupied the same relative positions as to the general desirability of the chips fried in them that they did for desirability of flavor of the chips with one exception, namely, in general desirability cottonseed oil came after peanut oil. Its average for effect on flavor was identical with that of hydrogenated cottonseed oil and below corn oil, although the difference was not significant.

The chips cooked in oils were preferred on the whole to those cooked in the lards. Of the lards the peanut and the hydrogenated gave better general results than the corn, rice, or prime steam. The

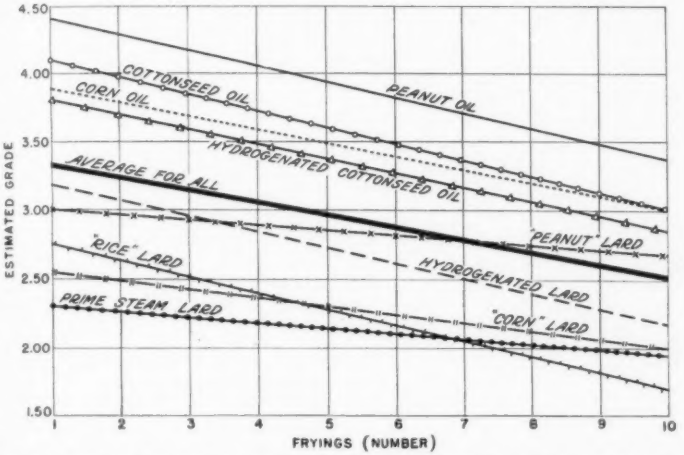

FIGURE 3.—Regression lines showing the estimated loss of grade for general desirability of chips fried in each of the nine fats during the 10 fryings of the two series combined.

differences among the last three were unimportant. An unweighted average for all eight factors, excluding general desirability, placed the fats in the same relative positions with the exception of prime steam lard, which ranked above corn and rice lard. This was evidently due to the influence of the score for color, as prime steam lard as well as cottonseed oil was second highest. As stated before, however, tests of significance indicated that the slight differences between the means of the prime steam, corn, and rice lards were probably due to chance and that the judges were unable to discern any consistent difference among them. It may be considered that the judgments for general desirability gave an accurate estimate of general effect of the fats on the chips.

Changes in Fats During Use as Evidenced in the Chips

There was some deterioration in the general quality of the chips as the number of fryings increased (fig. 3). Computations made after the chips had been judged for general desirability, showed that the

corn-lard, prime-steam-lard, and peanut-lard chips fell 0.04 to 0.06 of a grade per frying, and that all of the chips fried in the other fats fell 0.10 to 0.12 of a grade. The number of times the fats were used up to 10 did not affect the relative desirability of the chips materially. Chips fried in rice lard, which were in general superior to those fried in corn and prime-steam lard at the beginning of the tests, fell more rapidly in quality than the chips fried in the other lards, so that after the fifth frying they were inferior to those fried in corn lard, and after the seventh frying they were inferior to those fried in prime-steam lard. Chips fried in hydrogenated lard, which at the beginning were judged to be superior to those fried in peanut lard, were inferior to them after the third frying. Chips fried in cottonseed oil at the beginning were slightly superior to those fried in corn oil, but at the end of the tenth frying no difference could be discerned. Chips fried in peanut oil, which were held to be superior at the beginning of the tests, had about the same relative quality at the end of the tenth frying.

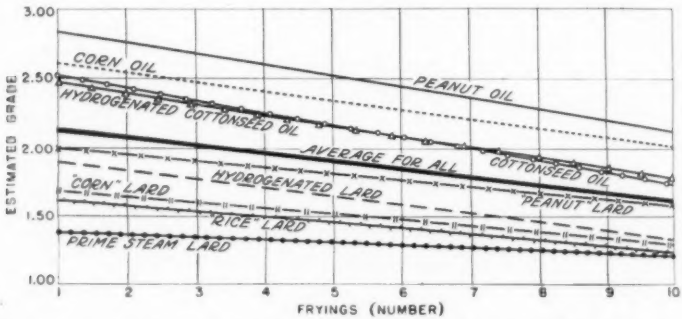

FIGURE 4.—Regression lines showing the estimated loss of grade for desirability of flavor of chips fried in each of the nine fats during the 10 fryings of the two series combined.

There was also a decrease in the desirability of flavor of the chips as the number of fryings increased (fig. 4). This trend was as follows: Cottonseed oil, peanut oil, and hydrogenated cottonseed oil fell 0.08 to 0.09 per frying; corn oil, hydrogenated lard, 0.06 to 0.07; peanut lard, rice lard, and corn lard, 0.04 to 0.05; prime-steam lard, 0.02. The flavor of the chips fried in the oils deteriorated somewhat more rapidly than those fried in the lards. However, at the end of the 10 fryings the chips fried in oil were still superior, although there was a slight tendency for the grades for flavor of the chips to converge as the fats were used repeatedly.

Effect of Storage on the Potato Chips

Organoleptic tests of chips fried in each of the fats and stored without light in white glassine bags at average room temperature and in a refrigerator indicated that low temperatures in most cases retarded the development of organoleptic rancidity (table 3). The storage temperature did not affect the crispness of the chip during the 60-day storage period.

TABLE 3.—*Effect of storage temperature on development of organoleptic rancidity and free fatty acids in potato chips fried in nine fats*

Fat used to fry potato chips	Period required for development of rancid flavor in chips when stored at—		Free fatty acid (as oleic acid)	
	Room temperature (22.6° C.)	Refrigerator temperature (3.2° C.)	Room temperature (22.6° C.)	Refrigerator temperature (3.2° C.)
	Days	*Days*	*Percent*	*Percent*
"Rice" lard	32	39	0.80	0.70
"Corn" lard	32	32	.49	.54
"Peanut" lard	25	39	.39	.34
Prime steam lard	32	39	.60	.52
Hydrogenated lard	32	39	.20	.18
Hydrogenated cottonseed oil	32	39	.19	.18
Cottonseed oil	32	46	.19	.15
Corn oil	46	46	.18	.15
Peanut oil	60	60	.24	.24

DISCUSSION OF RESULTS

In the present investigation low fat absorption was regarded as one of the criteria of a satisfactory fat for frying potato chips. Although the quantity of fat absorbed by the experimental chips was 6.9 percent higher than that reported by Woodruff and Blunt (*17*) and 7.3 percent higher than that reported by Atwater and Bryant (*1*) for commercial chips, the results agree with those of Woodruff and Blunt in that they were about the same for each of the different fats. Such findings are significant because the fats included those of both animal and vegetable origin, as well as those manufactured by different methods. The high fat absorption of the chips in this study may have been due to the lower frying temperature necessitated by the high sugar content of the potatoes.

From the standpoint of economy, a long frying life in a fat is desirable. The theory has been advanced (*9, 17*) that it is largely the long heating of fats which brings about their deterioration. The experimental fats were all heated to a temperature of 185° C. during the 10 fryings which totaled 8.5 hours, but the actual frying time was only 80 minutes. While none of the fats after 10 fryings showed a high free fatty acid content, the peroxide values were high enough, especially in the case of the lards, to make their fitness for use questionable. The large increase in free fatty acids in the fats extracted from chips of the tenth frying likewise brings into question the suitability of these fats and to some extent accounts for the poorer quality of the chips. There was no significant difference in the rate of deterioration of the fats from the first to the tenth frying, as shown by the judgments on the chips from each successive frying.

Frying fats are known to show an increase in acidity during use (*2, 5, 10, 11, 17*). The maximum amount (0.88 percent) found in this experimental work was well below the 2.0 percent at which objectionable flavor was reported by Porter, Michaelis, and Shay (*11*) when frying doughnuts.

A comparison of the chemical tests made upon the fats with judgments concerning the desirability of flavor of the chips showed that little if any relationship existed. While the chemical tests were consistent in showing the deterioration of a single fat they were of

little value in showing the differences between the kinds of fats. The different methods of manufacture may have had some bearing upon these findings (*17*).

King, Roschen, and Irwin (*8*) in their peroxide determinations, used a value of 20 millimols per kilogram as the point at which incipient organoleptic rancidity occurred in lards subjected to their accelerated stability test. They further noted that the rancid point was higher in the case of corn and cottonseed oils and of cottonseed-oil shortenings than in lards. These differences in the relation of rancid taste or organoleptic rancidity to peroxide content are probably due to variations in the relative speeds of the two reactions concerned in the development of rancidity (1) peroxide development and (2) formation of the compounds contributing to rancid flavor, discussed by Kilgore (*7*). It is probable that this variation in the relative speed of the two reactions is affected not only by the kind of fat but by the method of handling, and that it would be very much influenced by variations in the cooking process. Because of this it is impossible to state definitely the peroxide value at which any fat becomes rancid. It is likewise not possible to use the peroxide value as a basis for ranking the various fats in the present study as to their degree of rancidity. The general increase in peroxides showed that all the fats were in the process of developing rancidity. The differences between the two groups based on origin (animal or plant) appeared no greater than the differences within groups.

SUMMARY

Nine fats, including three kettle-rendered lards from animals fed on rations consisting largely of (1) peanut, (2) corn, and (3) brewer's rice, a standard prime steam lard, a hydrogenated lard, a hydrogenated cottonseed oil, and three highly refined oils from corn, cottonseed, and peanuts, respectively, were studied for use in the frying of potato chips. Frying technique was modified to permit of operations under controlled conditions. The results showed that fat absorption in the potato chips was about the same for all the fats used.

The chemical tests showed deterioration in all the fats. After 10 fryings, none of the fats had a high free fatty acid content, but the peroxide values were high enough, especially in the case of the lards, to make their fitness for use questionable. The percentage of free fatty acids in the fat extracted from the chips was greater than in the frying fat. No relationship was found between the chemical changes and the flavor of the chips.

The averaged values of the palatability tests made upon the potato chips showed that from the standpoint of general desirability, the oils were preferable for frying to the lards. Desirability of flavor was the most important item in forming the general opinion, and in the tests made peanut oil was judged to impart the most desirable flavor to the chips. In general desirability, cottonseed oil ranked next to peanut oil. Of the lards, peanut and hydrogenated were the best for frying chips. Further analysis indicated that the slight differences between the desirability of prime steam, corn, and rice, lards for frying were not significant.

Potato chips fried in the oils and in most cases those stored in the refrigerator kept fresh for the longest period.

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FASCIATION OF SWEET PEAS CAUSED BY PHYTOMONAS FASCIANS N. SP.[1]

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INTRODUCTION

The cause of fasciation in winter-flowering sweet peas (*Lathyrus odoratus* L.) has been studied because the continued loss from this disease in Ohio greenhouses has created a request for control measures. Fasciation has been reported by Brown (*1*)[2] as occurring near Washington, D. C., and by Muncie and Patel (*2*) in Iowa, who attribute its cause, respectively, to a weak or highly specialized strain of *Phytomonas tumefaciens* (Smith and Town.) Bergey et al. and to environmental or nutritional factors. This lack of agreement and the possibility that more than one agent or set of conditions might induce the disease suggested the advisability of investigating the cause in Ohio before seeking control measures.

SYMPTOMS

Many short, fleshy, thick, and aborted stems with misshapen leaves develop at or below the soil line on affected plants (fig. 1, *A*). These fasciated shoots originate either at the first or cotyledonary node, or at the second node of the stem (fig. 1, *B* and *C*). The mass of fasciated growth on old plants resembles a witches' broom and may reach a diameter of 3 inches, but usually does not extend over 1 or 2 inches above the ground; a normal green color develops in the portion exposed to the light. The main stem of an affected plant apparently grows normally, except that it is dwarfed and its productivity from the standpoint of marketable blossoms is reduced. Diseased plants seem to live about as long as normal individuals.

Certain symptoms, which are not usually found on naturally infected plants, may develop after artificial inoculation; for instance, if the young epicotyl is inoculated just as it emerges from the seed coat or if the organism is present in large numbers in the soil, a gall-like growth is produced sometimes and no main stem develops (fig. 1, *E*). When inoculations are made in the leaf axils of young plants some proliferation is evident after a month. The growth usually has the appearance of a multiple bud and occasionally one or more abnormal shoots will develop (fig. 1, *D*).

The writer has been unable to produce similar symptoms by inoculating sweet pea plants with the crown gall organism. Typical crown galls developed in the leaf axils (fig. 1, *F*) and on the lower part of the stem (fig. 1, *G*) after inoculations were made with *Phytomonas*

[1] Received for publication Mar. 12, 1936; issued October 1936. Since this paper was submitted for publication ar article by Margaret S. Lacey has appeared in The Annals of Applied Biology, vol. 23, pp. 302 to 310, entitled "Studies in Bacteriosis. XXII. I. The Isolation of a Bacterium Associated with 'Fasciation' of Sweet Peas, 'Cauliflower' Strawberry Plants and 'Leafy Gall' of Various Plants." A preliminary description of the organism is given which seems to identify it as the bacterium described in the present paper. No name is suggested for the organism.

[2] Reference is made by number (italic) to Literature Cited, p. 394.

tumefaciens. Muncie and Patel (*2*) have reported similar results. Smith (*5*), however, has pointed out that fasciation can be produced by inoculating *P. tumefaciens* into the leaf axils of a variety of plants.

ISOLATION AND INOCULATION STUDIES

Some preliminary experiments were made to find out whether or not the disease is infectious as it occurs in Ohio. Fifty seeds of the Ball's Orange variety of sweet peas were treated in a 5-percent solution of formaldehyde for 5 minutes and rinsed in sterile water. Twenty-five were planted in 3-inch pots of sterilized soil, five to a pot. The other 25 were rubbed in the crushed fasciated tissue from the base of a diseased plant and then planted in pots of sterile soil. Twenty plants grew in the control lot, and 6 weeks after planting all 20 were healthy; whereas 8 of the 22 plants from the seed rubbed in the crushed tissue showed fasciation.

Formaldehyde-treated seed was planted in soil taken from a greenhouse bed where most of the sweet peas were fasciated. Another portion of the same soil was sterilized by autoclaving before the seed was planted. Thirteen plants, eight of which showed fasciation 6 weeks after planting, grew in the unsterilized soil; whereas 12 plants, 1 of which was fasciated, grew in the sterilized soil.

These two preliminary tests, supplemented by observations in commercial greenhouses where sweet peas were grown, seemed to indicate that the disease in some instances at least was infectious. Since Brown (*1*) had reported that fasciation of sweet peas may be caused by the crown gall bacterium, an effort was made to isolate this organism from sweet peas showing the disease. The dilution plate method was used with potato-dextrose agar, nutrient agar, and Patel's (*3*) medium. The plates were incubated for 3 or 4 days, and all colonies resembling *Phytomonas tumefaciens* were transferred and used in inoculation tests. Numerous attempts were made during 1933 and 1934 to isolate the crown gall organism from fasciated sweet peas, and about 80 selected colonies were tested for pathogenicity. In all instances negative results were obtained. Occasionally, fasciated plants developed but they appeared just as often among the control plants as among those which had been inoculated.

In these early experiments a particular lot of seed was used which always produced some fasciated plants, even though the seed was treated by shaking for 5 minutes in a 5-percent solution of formaldehyde. The seed coat of most varieties of sweet peas is wrinkled and more or less pitted. Numerous small air bubbles arise from these pits and cling to the seed when it is submerged in a water solution of a disinfectant, indicating that the surface is not thoroughly wetted. In the later inoculation tests the seed was immersed in alcohol for 1 minute and then in 1–1,000 $HgCl_2$ for 20 minutes. After this treatment, the control plants were always free of fasciation. The disease is probably seed-borne to some extent.

Finally, in a set of potato-dextrose agar plates which had been incubated for 1 week, a few orange-colored bacterial colonies about 1 mm in diameter were noticed. Since these were distinctly different from any colonies observed previously, they were transferred to agar slants and to broth. On July 31, 1934, this organism, along with several others which had been saved, was used to inoculate

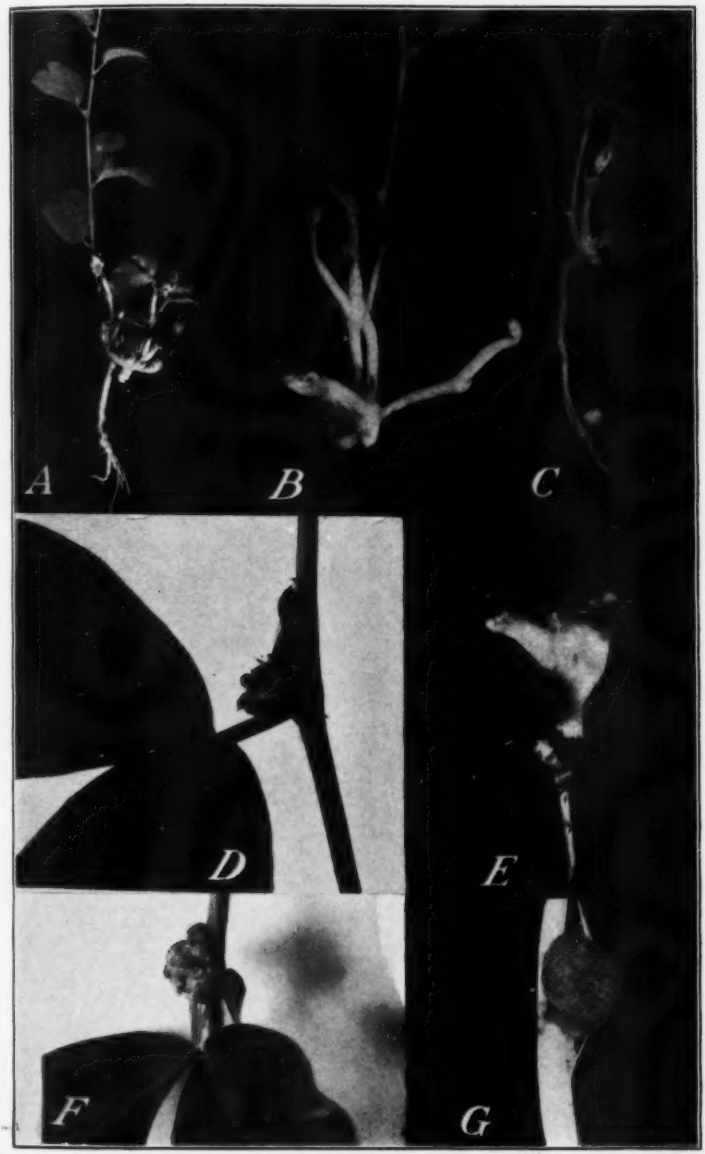

FIGURE 1.—*A*, Sweet pea plant 3 months old showing fasciation; *B*, fasciation originating at the cotyledonary node; *C*, fasciation originating at the second node; *D*, 1 month after inoculation at leaf axil; *E*, epicotyl develops into a gall-like structure and sometimes no main stem forms if the organism is very abundant in the soil; *F*, result of inoculation at leaf axil with *Phytomonas tumefaciens*; *G*, result of inoculation on lower part of stem with *P. tumefaciens*.

seed of the Ball's Orange variety. Inoculations were made by pouring 24-hour-old broth cultures of the organisms over the seeds when they were planted. On September 4 the experiment was discontinued, and three plants out of five in the pot where the seed was inoculated with the orange bacterium showed fasciation. No fasciated plants occurred in any of the other pots, and of a total of 10 control plants all were normal. Apparently the same organism that had been used to inoculate the seed was reisolated from the fasciated plants.

This same organism has been isolated by the poured-plate method from diseased peas found growing in six different Ohio greenhouses. The inoculation and reisolation tests with these six isolates are summarized in table 1. The best results in making isolations have been obtained by taking a large piece of tissue from the base of the fasciated growth, dipping it in alcohol, flaming it, and then crushing it in about 5 cc of sterile water. After 2 to 4 hours one drop of the liquid is transferred with a sterile pipette to a sterile dish, two drops are placed in a second dish, and three drops in a third. Another set of three plates is prepared in exactly the same way. The plates are poured with melted potato-dextrose agar and incubated at 25° C. After 7 days the orange-colored colonies, about 1 mm in diameter, are easily detected. Sometimes as many as 10 to 12 appear in a single plate, more often the number is from 1 to 5, and in some plates none develop.

TABLE 1.—*Results of inoculating sweet peas* [1] *with poured plate cultures of the fasciation-producing bacteria*

Date of inoculation	Source of culture	Inoculations [2]	Fasciated	Controls [3]
1934		*Number*	*Number*	*Number*
Aug. 25	Wooster, Ohio, culture A [4]	18	12	20
Sept. 18	Wooster, Ohio, culture A1	24	23	25
Dec. 10	Reisolation of culture A1	10	3	17
Oct. 29	Wooster, Ohio, culture B [5]	5	1	5
Do	Wooster, Ohio, culture B1	5	4	5
Dec. 10	Reisolation of culture B1	11	4	17
Oct. 29	Barberton, Ohio, culture C	4	3	5
Do	Barberton, Ohio, culture C1	2	1	5
Dec. 10	Reisolation of culture C1	11	5	17
Oct. 29	Wooster, Ohio, culture D [6]	5	1	5
Do	Wooster, Ohio, culture D1	6	3	5
Dec. 10	Reisolation of culture D1	10	4	17
Nov. 8	Medina, Ohio, culture E	5	4	5
Do	Medina, Ohio, culture E1	5	3	5
Do	Medina, Ohio, culture E2	5	2	5
Do	Medina, Ohio, culture E3	5	3	5
Dec. 10	Reisolation of culture E	10	7	17
Do	Reisolation of culture E1	9	4	17
Nov. 14	Ashland, Ohio, culture F	3	1	5
Do	Ashland, Ohio, culture F1	5	1	5
1935				
Apr. 8	Reisolation of culture F1	6	3	30

[1] Variety, Ball's Orange.
[2] Inoculations were made by pouring a broth culture 24 to 48 hours old over the seeds at the time they were planted. 5 seeds were planted in 3-inch pots of sterilized soil placed on a greenhouse bench. Each pot rested on a block of wood to prevent contamination from 1 pot to another by drainage water. Pots and soil were sterilized by autoclaving.
[3] None of the controls became fasciated.
[4] From horticultural greenhouse.
[5] From botanical greenhouse.
[6] From Dewitt greenhouse.

Single-cell isolates [3] were made from all six of the original cultures. The methods employed in isolating and cultivating the single bacterial cells were essentially those described by Wright, Hendrickson, and Riker (7). All 15 of the single-cell isolates were tested for pathogenicity, and the results are summarized in table 2.

The organism was obtained from many of the plants inoculated with reisolations from diseased plants which had been inoculated with original cultures. These reisolates appeared to be identical with the original isolates when grown in pure culture. This fact, together with the results of inoculations given in tables 1 and 2, is thought to be abundant proof that the organism is pathogenic and capable of causing fasciation in sweet peas. It is believed to be an undescribed species.

TABLE 2.—*Results of inoculating sweet peas with single-cell isolates of the fasciation-producing bacteria*

Date of inoculation	Source of culture [1]	Inoculations [2]	Fasciated	Controls [3]
1935		*Number*	*Number*	*Number*
Apr. 8	Culture AA, single-cell isolate from culture A1	9	6	30
	Culture AA1, single-cell isolate from culture A1	8	6	30
	Culture BB, single-cell isolate from culture B1	8	6	30
	Culture CC, single-cell isolate from culture C1	5	2	30
	Culture CC1, single-cell isolate from culture C1	11	8	30
	Culture CC2, single-cell isolate from culture C1	7	2	30
	Culture CC3, single-cell isolate from culture C1	6	5	30
	Culture CC4, single-cell isolate from culture C1	6	3	30
	Culture DD, single-cell isolate from culture D1	5	1	30
	Culture DD1, single-cell isolate from culture D1	4	0	30
	Culture EE, single-cell isolate from culture E	7	3	30
	Culture FF, single-cell isolate from culture F1	21	7	30
	Culture FF1, single-cell isolate from culture F1	7	1	30
	Culture FF2, single-cell isolate from culture F1	11	5	30
	Culture FF3, single-cell isolate from culture F1	9	5	30
May 8	Culture DD1, single-cell isolate from culture D1	10	7	30
July 3	Reisolation of culture AA, inoculation of May 8 [4]	7	2	18
	Reisolation of culture AA1, inoculation of May 8 [4]	6	5	18
Oct. 2	Reisolation of culture BB, inoculation of May 8 [4]	11	10	18
	Reisolation of culture BB, inoculation of July 3	40	23	45
July 3	Reisolation of culture CC, inoculation of May 8	9	1	18
	Reisolation of culture CC1, inoculation of May 8	8	2	18
	Reisolation of culture CC3, inoculation of may 8	7	4	18
	Reisolation of culture CC4, inoculation of May 8	6	5	18
	Reisolation of culture DD1, inoculation of May 8	8	6	18
	Reisolation of culture FF, inoculation of Apr. 8	10	9	18
	Reisolation of culture FF1, inoculation of May 8	11	10	18

[1] See table 1.

[2] The inoculations were made from broth cultures 48 hours old by using sterile pipettes drawn to a sharp point. The seedlings (4 or 5 days old) were pricked at the cotyledonary nodes with the pipettes while drops of the inoculum flooded the area. Controls were treated in the same way except that sterile water replaced the inoculum.

[3] None of the controls became fasciated.

[4] The May 8 inoculations are not given, since they were a duplication of those of Apr. 8 and the results were similar.

HOST RANGE

No systematic effort has been made to determine the host range of the organism, but the results obtained by inoculating a few different plants indicate that susceptibility is not confined to a single genus. Twenty-five garden pea seeds (variety Premium Gem) were inoculated at the time of planting in a flat of sterilized soil. Nineteen plants grew, and 1 month later 17 showed symptoms of fasciation (fig. 2, *A*). The organism was recovered from these plants and used

[3] This work was done in the plant-pathology laboratory at the University of Wisconsin, and the writer is indebted to Dr. A. J. Riker for the use of equipment and for much helpful advice.

to inoculate sweet peas which later became diseased. The writer has never observed fasciation on garden peas growing out of doors, but Brown (*1*) reported that a few plants of this kind affected with the disease had been sent to Washington from Maryland and Virginia.

Twenty-five seeds each of 10 commercial varieties [4] of winter-flowering sweet peas were sterilized and inoculated at the time of planting in a flat of sterilized soil. Five weeks later the plants were removed and examined for fasciation. All 10 varieties were found

FIGURE 2.—*A*, Garden pea (variety Premium Gem) 1 month after inoculation; *B*, fasciation at the base of petunia plant developing after inoculation; *C*, chrysanthemum naturally infected.

susceptible to the disease to about the same extent. An average of 64 percent of the plants was fasciated.

A mass of fasciated shoots developed at the base of petunia (fig. 2, *B*) tobacco (variety Quesnel), and *Gypsophila paniculata* plants after inoculation by the needle-prick method. The organism was isolated from a fasciated growth at the base of a geranium from Canton, Ohio, and from a chrysanthemum received from Dr. R. P. White of New Jersey. Geranium plants were later inoculated with the organism and developed symptoms of the disease. Many plants of several varieties of chrysanthemums in one Ohio greenhouse were fasciated

[4] Ball's Orange, Ball's Rose, Harmony, Ball's Blue, New Majestic Rose, New Hope, Jeanne Mamitsch, Snow Storm, Mrs. Herbert Hoover, and White Harmony.

at the base when they were removed from the beds (fig. 2, *C*). The organism was isolated from some of these plants, and in this particular greenhouse fasciation has been especially common on winter and spring crops of sweet peas following chrysanthemums.

In a single experiment potato, tomato, aster, verbena, calendula, gaillardia, snapdragon, canterbury-bell, zinnia, marigold, and bean plants were inoculated at the base of the stem but negative results were obtained.

THE CAUSAL ORGANISM[5]

Unless otherwise indicated the methods given by the Society of American Bacteriologists (*6*) have been followed in making the determinations described below. Each of the tests was run in duplicate or triplicate. In most cases the tests were made three different times, usually with all 15 of the single-cell isolates and some of the original cultures.

CULTURAL CHARACTERISTICS

Three of the six original isolates were of the rough colony type, whereas the other three were smooth. Six of the fifteen single-cell isolates were of the rough type and have remained so in culture. Both rough and smooth types were obtained from the same culture in one instance when single cells were isolated. Rough colony types have been isolated from plants inoculated with a single-cell isolate which has consistently been the smooth type in culture.

The cultural characteristics of the organism grown on and in several culture media incubated at a temperature near 25° C. were as follows: On potato-dextrose agar plates minute, light cream-colored colonies were visible on the surface after 72 hours. After 7 days the colonies were mostly punctiform, but some were circular with the largest not over 3 or 4 mm in diameter. Submerged colonies were barely visible. The color after 1 week was cadmium yellow to deep chrome or orange buff (*4*). Rough colonies were near cadmium yellow, whereas the smooth type was more nearly deep chrome or orange buff.

On nutrient agar [6] plates the growth was much slower than on potato-dextrose agar, and the color was cream.

After 3 days on potato-dextrose agar slants the growth was moderate, filiform, raised, dull to glistening, and rugose in the case of rough colonies, glistening and smooth with smooth colonies, opaque, cadmium yellow to deep chrome in color, and butyrous in consistency. No odor was detected and the medium was not changed in appearance. After a week the characteristics were the same except that abundant growth was present.

On nutrient agar slants after 1 week the growth was moderate, filiform, flat, dull to glistening, smooth, opaque, cream-colored, and butyrous. There was no visible change in the media and no odor.

Scarcely any clouding of nutrient broth [7] was visible after 24 hours, but after 48 hours broth was slightly clouded and a thin, fragile pellicle with a distinct rim had formed.

After 7 days in Fermi's solution the medium was slightly clouded and a sparse, fragile pellicle with a rim had formed on the surface.

[5] The author is indebted to Roy C. Thomas of this station for advice and for many helpful suggestions in interpreting the results of the bacteriological studies.

[6] Difco nutrient agar, pH 7.4.

[7] Difco nutrient broth, pH 6.6.

Uschinsky's solution was slightly clouded after 7 days, a pellicle similar to that in Fermi's solution had formed, but the growth was a little more abundant than in the latter.

In Cohn's solution growth was not as abundant after 7 days as in either Fermi's or Uschinsky's medium. Scarcely any clouding was visible, but in most instances a very fragile pellicle had formed.

MORPHOLOGY

When grown on potato-dextrose agar the organism was a rod with pointed to rounded ends, usually growing singly but sometimes in pairs or even short chains of three or four cells. Occasionally a pair of cells, probably in the act of dividing, formed a U or V. Grown on this medium for 18 to 24 hours at 25° C. and stained with Gram's stain, the size was 1.5μ to 4.0μ long and 0.5μ to 0.9μ wide. The average measurement of 50 individuals was 2.7μ by 0.69μ. This average was obtained by measuring 10 individuals of each of 5 strains by the use of a filar ocular micrometer.

No evidence of motility has been observed on potato-dextrose agar or in peptone-salt-dextrose liquid medium. Many hanging drops have been prepared and studied in addition to those observed while making single-cell isolates. Flagella could not be demonstrated with Casares-Gil's flagella stain.

Cultures 24 and 48 hours and 5, 8, and 42 days old growing on potato-dextrose agar were examined for capsules by Anthony's method. The formation of capsules could not be demonstrated in any of the rough colony type of single-cell isolates. In the smooth type, however, what appeared to be capsules were found in the cultures which were 8 and 42 days old. Younger cultures of this type showed no evidence of capsules.

Attempts to demonstrate spores in potato-dextrose agar cultures which were 3, 10, and 12 weeks old gave only negative results. Heavy suspensions of the organism were prepared in sterile water and heated to 85° C. for 10 minutes in glass ampules. No growth developed when transfers were made to nutrient broth. In material from cultures which were 1, 2, 4, and 8 weeks old stained by Dorner's method, no spores were found. One, two, and sometimes three small round bodies, however, were observed in the bacterial cells.

PHYSIOLOGICAL CHARACTERS

LIQUEFACTION OF GELATIN.—No liquefaction occurred in gelatin stabs incubated at 20° C. for 7 weeks. In the same tests *Phytomonas phaseoli* (E. F. S.) Bergey et al. used for comparison produced strong liquefaction. A negative test for liquefaction of gelatin was also obtained by using Frazier's gelatin medium. *P. phaseoli* gave a positive test, whereas a negative test was obtained with *Phytomonas stewarti* (E. F. S.) Bergey et al. by this method.

LITMUS MILK.—After 48 hours in litmus milk slight bluing occurred at the top of the milk column. The blue color was distinct after 5 days and the litmus was slightly reduced in the bottom of 16-mm tubes containing about 8 cc of the medium. At the end of 1 month the milk was blue to within one-half inch of the bottom of the tubes where the litmus was reduced. No curd formed and the milk appeared unchanged except as noted. A pellicle consisting of

clumps of the organism, cadmium yellow in color, formed on the surface. The milk in the control tubes after 1 month was a uniform lavendar throughout.

REDUCTION OF NITRATES.—Nitrates were reduced to nitrites. Tests were made on synthetic nitrate agar and on peptone beef-extract nitrate agar by using sulphanilic acid and alpha-naphthylamine reagents. *Escherichia coli* (Escherich) Castellani and Chalmers and *Phytomonas phaseoli* were used for comparison. After 24 hours a faint but distinct test for nitrites was obtained in the synthetic medium. After 48 hours and after 1 week nitrites were found in the synthetic nitrate agar, but no evidence of nitrites appeared in the peptone beef-extract nitrate agar. In no case was the red color as intense in the tubes of synthetic medium inoculated with the organism in question as with *E. coli*. In every test with both media the control tubes, as well as those inoculated with *P. phaseoli*, showed no evidence of nitrites.

Ammonia was not found by the Hansen test in synthetic nitrate agar but ammonia was present in cultures on peptone beef-extract nitrate agar after they had reached an age of 2 and 7 days.

INDOLE PRODUCTION.—No indole was produced. Bacto-tryptone cultures 2, 4, 12, and 28 days old all gave negative tests by the Goré modification of the Ehrlich-Böhme method. A positive test for indole was obtained with *Escherichia coli* and a negative test with *Phytomonas phaseoli*.

FERMENTATION OF CARBOHYDRATES.—Three different tests were made with each carbon source. (1) Synthetic carbohydrate medium[8] with bromcresol purple indicator was prepared so that it contained 1 percent of the carbon source and 1.5 percent of agar. The carbon source was added before autoclaving. (2) The synthetic carbohydrate medium without agar was tubed in test tubes containing Durham tubes and sterilized. The carbon source, if soluble, was sterilized separately by passing through a Chamberland-Pasteur L3 filter under a suction of 5 to 10 inches of mercury. It was then transferred aseptically with pipettes to the tubes of medium in amount sufficient to bring the concentration to 1 percent. Media prepared in this way were incubated for 1 week before using, and if any tubes showed contamination they were discarded. (3) Synthetic carbohydrate liquid media were prepared in the manner just indicated, except that bromcresol purple indicator was added.

An increase in the hydrogen-ion concentration was detected in the case of the indicator media by the change in color of the indicator as compared to uninoculated control tubes. Any change in the hydrogen-ion concentration of the nonindicator media was determined by comparing the pH of inoculated with uninoculated tubes of the media. The pH measurements were made electrometrically with a potentiometer, quinhydrone electrode, and calomel half cell.

All 15 of the single-cell isolates, as well as some of the original isolates, were used with the less expensive carbon sources. With the more expensive sugars the tests were made with only five of the single-cell isolates.

No gas appeared in the Durham tubes in the liquid cultures with any of the carbon sources.

[8] Monobasic ammonium phosphate, 1.0 g; potassium chloride, 0.2 g; magnesium sulphate, 0.2 g; water, 1,000 cc; carbon source, 10.0 g.

The hydrogen-ion concentration was increased in media containing dextrose, sucrose, maltose, dextrin, glycerol, mannitol, arabinose, xylose, galactose, mannose, and levulose; whereas the reaction did not change when lactose, inulin, rhamnose, raffinose, and starch were used as sources of carbon. In no instance were the results different when the carbon source was autoclaved than when it was sterilized by filtration.

The hydrogen-ion concentration produced from different carbon sources by five of the single-cell isolates after an incubation of 1 week in synthetic carbohydrate medium is given in table 3.

TABLE 3.—*Hydrogen-ion concentration produced from various carbon sources by single-cell isolates of the fasciation-producing bacteria after an incubation of 7 days in synthetic carbohydrate media at room temperature*

Source of carbon	AA	CC4	DD	EE	FF	Control
	pH	*pH*	*pH*	*pH*	*pH*	*pH*
Dextrose	6.8	6.7	6.65	6.85	6.65	7.0
Sucrose	6.5	6.25	6.15	6.3	6.2	6.95
Maltose	6.65	6.65	6.65	6.65	6.5	6.9
Dextrin	6.65	6.55	6.6	6.65	6.65	6.7
Glycerol	6.7	6.6	6.7	6.7	6.7	6.9
Mannitol	6.5	6.5	6.5	6.4	6.6	6.9
Arabinose	6.45	6.1	6.5	6.35	6.35	6.9
Xylose	6.7	6.7	6.8	6.75	6.85	7.0
Galactose	6.8	6.7	6.8	6.9	6.8	7.0
Mannose	5.6	5.4	4.5	5.6	4.5	6.9
Levulose	6.85	6.65	6.5	6.7	6.7	7.0
Lactose	6.75	6.85	6.8	6.85	6.8	6.85
Inulin	6.7	6.7	6.7	6.7	6.7	6.7
Rhamnose	6.95	6.95	7.0	7.0	7.0	7.0
Raffinose	6.85	6.85	6.85	6.85	6.85	6.85
Starch	7.0	7.0	7.0	7.0	7.0	7.0

DIASTATIC ACTION.—Starch was not reduced. On starch-agar plates streaked with the organism and incubated for 7 days at 25° C. no clearing was evident when the plates were flooded with iodine.

HYDROGEN SULPHIDE PRODUCTION.—Hydrogen sulphide was produced. All single-cell isolates were used to inoculate Difco lead acetate agar slants and lead acetate papers were suspended in the tubes. After 3 days very little change could be detected in the agar, but the test papers were blackened at the tip in all instances and as much as 1 inch back from the tip in some cases. The organism produced more H_2S than was formed by *Phytomonas phaseoli*, as was indicated by the extent of the blackening of the test papers.

TOLERATION OF SODIUM CHLORIDE.—The organism grew in nutrient broth containing sodium chloride up to 8 percent. Ten percent practically inhibited growth. The growth in broth containing 6, 7, and 8 percent of sodium chloride consisted almost entirely of a fragile pellicle on the surface and a rim on the walls of the tube.

OXYGEN RELATIONS.—The organism was aerobic. In shake cultures a heavy growth developed on the surface of potato-dextrose agar, but no colonies formed in the agar at depths greater than one-eighth of an inch of the surface. When long sterile cover slips were placed on agar plates streaked with the organism no growth occurred under the cover except at the edge. Melted agar suspensions of the organism were drawn into sterile glass tubes which were then sealed. Very weak growth, which was light in color, occurred at the ends of the agar column but none at all in the agar. When potato-dextrose agar

in bottles which could be sealed with airtight caps was inoculated, the growth was weak and light in color in contrast to the heavy growth of cadmium yellow when the bottles were plugged with cotton.

THERMAL RELATIONS.—The exact temperature limits for growth of the organism were not determined. Growth occurs, however, from 7° to 35° C. with the optimum at 25° to 28°.

Freshly made transfers on potato-dextrose agar were stored for 5 weeks at a temperature of −25° C. All showed typical growth after 7 days at room temperature.

The thermal death point varied from 55° to 57° C. when fresh nutrient broth cultures in thin-walled tubes were exposed in a water bath for 10 minutes.

VIRULENCE.—The organism has retained its virulence in culture on potato-dextrose agar for over 18 months.

STAINING REACTIONS

Tests made by using Hucker's modification of Gram's stain showed the organism to be Gram positive. Smears from cultures 24 to 96 hours old, growing on potato-dextrose agar and beef-extract agar, were stained between smears of *Phytomonas phaseoli* and *P. flaccumfaciens* (Hedges) Bergey et al. In cultures 6 weeks old several cells stained Gram negative. Also in cultures of this age round bodies (sometimes one, two, or three in a cell) were found which stained darker than the rest of the cell.

The organism was not acidfast. Potato-dextrose agar cultures 48 hours old were treated by Ziehl-Neelsen's method.

TECHNICAL DESCRIPTION

Phytomonas fascians[9] n. sp.

Nonmotile rod 1.5μ to 4.0μ long, 0.5μ to 0.9μ wide, average 2.7μ by 0.69μ. Spores not formed. Gram-positive but not acid-fast. Optimum growth at 25° to 28° C.; thermal death point 55° to 57°. No diastatic action. Gelatin not liquefied but hydrogen sulphide formed. Alkalinity produced in litmus milk and litmus reduced in bottom of tubes; otherwise litmus milk unchanged. Organism aerobic. On potato-dextrose agar cadmium yellow to deep chrome or orange-buff. Nitrates reduced to nitrites. Ammonia produced; no indole formed. Hydrogen-ion concentration increased but no gas found when organism was grown on synthetic carbohydrate medium with following carbon sources: Dextrose, sucrose, maltose, dextrin, glycerol, mannitol, arabinose, xylose, galactose, mannose, and levulose. Gas not found and hydrogen-ion concentration not increased in synthetic carbohydrate medium with lactose, inulin, starch, rhamnose, and raffinose as carbon sources. Slight turbidity with sediment forms in broth and thin fragile pellicle with rim develops on surface. Distinct but very fragile pellicle with rim forms on Fermi's and Uschinsky's solutions; very slight clouding of media. Growth in Cohn's solution not as good as in Fermi's or Uschinsky's. Organism causes development of fasciated growth at base of sweet peas and certain other plants.

DISCUSSION

The fasciation-producing bacterium is another organism that induces cell stimulation in some species of plants. Galls or tumors, such as are caused by *Phytomonas tumefaciens*, *P. beticola* (Smith,

[9] This name was selected in consultation with Prof. F. H. Cowles of the College of Wooster and was first published in a preliminary note in the Fifty-fourth Ann. Rept. Ohio Agr. Expt. Sta. Bull. 561, p. 39, January 1936.

Brown, and Town). Bergey et al., *P. savastanoi* (E. F. S.) Bergey et al., *P. savastanoi* var. *nerii* C. O. Smith, and *P. gypsophilae* (Brown) Bergey et al. are not formed. Stimulation by this organism normally causes numerous fasciated shoots to develop from the base of the stem of the host plant. Apparently, infection does not occur on the roots, and in no instance have any abnormalities resembling those caused by the hairy root organism (*P. rhizogenes* Riker et al.) been observed on diseased plants.

SUMMARY

The cause of fasciation of winter-flowering sweet peas in Ohio has been investigated and found to be a bacterium.

Pathogenic isolates of the organism have been obtained from six different sources. Fifteen single-cell isolates were made from the original cultures and all of these were pathogenic.

Fleshy, fasciated shoots developed at the base of garden peas, petunias, geraniums, tobacco plants, *Gypsophila paniculata* plants, and 10 varieties of winter-flowering sweet peas when they were inoculated with the bacteria. In addition to these hosts, the organism has been isolated from fasciated growths at the base of chrysanthemum plants.

Bacteriological studies are described and the organism is named *Phytomonas fascians* n. sp.

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SPECIFIC EFFECTS OF ZINC APPLICATIONS ON LEAVES AND TWIGS OF ORANGE TREES AFFECTED WITH MOTTLE-LEAF[1]

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INTRODUCTION

This paper presents data on the effects of zinc salts on the growth of leaves and twigs of orange trees affected with mottle-leaf.[2] Trees in an orchard near Riverside, Calif., were selected for observation. They were about 30 years old at the time of treatment and had been severely affected with mottle-leaf for many years. Although the trees had produced a certain amount of new growth each year, the leaves were mottled and dwarfed. Many of the shoots had died back, and the quantity of fruit produced was subnormal.

In March 1934, a short time before the appearance of the first cycle of growth, the trees were sprayed with a mixture containing 10 pounds of zinc sulphate (commercial) and 5 pounds of hydrated lime, in 100 gallons of water. A few weeks after the application of the spray material the trees began to show improvement in the amount and character of the spring growth. This improvement has been maintained during the two following seasons, while untreated trees in adjacent rows have remained unthrifty and have borne typically mottled foliage. Other trees were similarly sprayed in October 1934 and showed normal growth the following spring.

The old, dwarfed leaves on the treated trees did not grow to normal size or shape after treatment, but they produced chloroplasts and assumed the green color of normal leaves. Apparently the leaf cells had lost their meristematic character insofar as multiplication was concerned, but the mitochondria proceeded to develop into plastids which synthesized chlorophyll.[3]

On treated trees it was possible to find in May 1935 many representative twigs bearing the small, elliptic-lanceolate leaves (fig. 1) characteristic of unsprayed mottled trees and also bearing normal green leaves of the 1934 cycles of growth. No such contrasts were observable on the untreated trees. The marked transition from dwarfed to normal leaves was coincident with the production of the first cycle of growth after the application of the spray mixture containing zinc sulphate and lime. This phenomenon indicates that zinc was quickly absorbed by the tree and promptly affected the metabolism of the organs which suffered from the systemic disease mentioned. The application of zinc sulphate to the soil has been

[1] Received for publication Mar. 26, 1936; issued October 1936. Paper no. 350, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, Calif.

For detailed descriptions of the symptoms of affected trees, see the following: FAWCETT, H. S., and LEE, H. A. CITRUS DISEASES AND THEIR CONTROL. 582 pp., illus. New York and London. 1926.

[2] REED, H. S., and DUFRÉNOY, J. THE EFFECTS OF ZINC AND IRON SALTS ON THE CELL STRUCTURE OF MOTTLED ORANGE LEAVES. Hilgardia 9: 113–141, illus. 1935.

followed by similar improvements in growth, though the time required for expression has been longer, and injury has resulted in some cases.[4]

OBSERVATIONS ON TWIGS AND LEAVES

The twigs shown in figure 1 were photographed in May 1935. The differences between the leaves produced before and after the

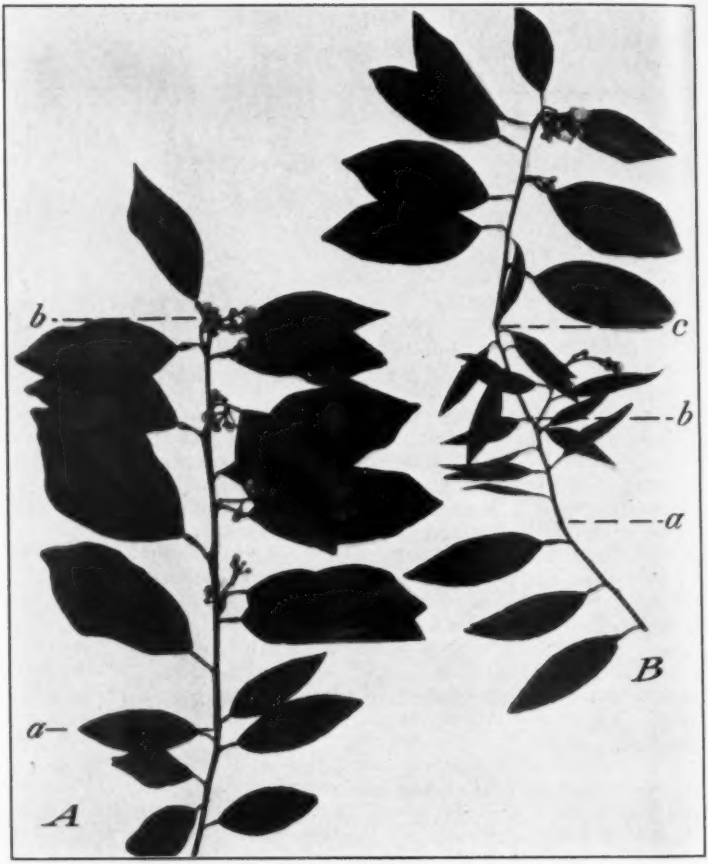

FIGURE 1.—Valencia orange twigs showing the effects of zinc-lime spray on trees affected with mottle-leaf: *A*, Cycle of growth below *a* produced small leaves before the tree was sprayed in October 1934; the cycle *a-b* was produced in the autumn of 1934 and bore large leaves; *B*, *a-b* and *b-c* show the cycles of growth and the small leaves borne before the tree was sprayed; the cycle above *c*, bearing large leaves, was produced after the tree was sprayed in May 1934. Both samples were collected in May 1935.

zinc sulphate-lime mixture was applied are striking. The shoot *a-b-c* in figure 1, *B*, was cut from a tree which had been sprayed in May 1934. During the remainder of the summer it produced one

[4] PARKER, E. R. EXPERIMENTS ON THE TREATMENT OF MOTTLE-LEAF OF CITRUS TREES. Amer. Soc. Hort. Sci. Proc. 31 (sup. vol.): 98–107. 1934.

cycle of growth, starting at the point *c;* this cycle was longer than earlier ones and bore healthy leaves. The greater length of the internodes of the healthy cycle is characteristic of normal growth. The other shoot shown in figure 1, *A*, produced one cycle of growth, *a-b*, after it was sprayed with the zinc sulphate-lime mixture in October 1934. It also bore healthy, oval leaves, which were much larger than those of the preceding cycle. The healthy twigs produced flowers at many of the nodes, in contrast to the few flowers on twigs of the preceding cycles.

The average area of leaves from affected trees gives a more precise indication of the effects of the spray solution containing zinc sulphate. Data were obtained from leaves collected April 15, 1935, from trees which had been sprayed in May 1934, and from comparable unsprayed trees. Each sample consisted of 100 mature leaves selected from the four sides of the trees. The outlines of the leaves were traced on paper and measured with a planimeter. The mean areas of the small leaves on the cycle of growth immediately preceding the treatments were very similar, 0.74 ± 0.01 and 0.75 ± 0.01 square inch for the untreated and for the sprayed trees, respectively. The leaves produced on the late summer or fall cycle of growth were larger on both the control and the treated trees, but the increase in size on the sprayed trees was striking, the figures being 1.61 ± 0.02 and 3.67 ± 0.02 square inches, respectively. The statistical reliability of the means is evident by comparison with their probable errors.

The width of phloem and xylem tissues in twigs may also serve as an index of their growth and hence express the effect of zinc sulphate on cellular activity, at least in respect to the cambium.

On May 7, 1935, twigs were selected from sprayed and unsprayed trees. As in the collection of the leaves, care was taken to obtain representative samples. Only twigs of an ascertainable age were chosen. These were of three ages: (1) Twigs of the 1935 spring cycle; that is, new growth; (2) twigs of the 1934 spring cycle, which had grown approximately 14 months; and (3) twigs of the 1933 spring cycle, which had grown approximately 26 months.

Twenty to thirty twigs were cut to convenient lengths and placed in a 1 : 1 mixture of water and glycerin, where they remained for several weeks. Transverse sections were then cut on a sliding microtome, stained with safranine, washed, and mounted on slides for measurement.

TABLE 1.—*Effect of zinc sulphate-lime spray on the average width of phloem and xylem tissues of orange twigs*

Designations of the twigs (see text)	Average width (microns) of—			
	Phloem in—		Xylem in—	
	Unsprayed twigs	Twigs sprayed May 1934	Unsprayed twigs	Twigs sprayed May 1934
1, 1935 spring cycle (March to May 1935)	127.8±2.3	113.8±2.0	119.6± 5.7	124.3± 3.8
2, 1934 spring cycle (March 1934 to May 1935)	199.7±5.4	187.3±4.9	381.3±14.5	518.5±14.8
3, 1933 spring cycle (March 1933 to May 1935)	249.0±8.0	218.9±5.0	630.8±18.1	716.4±17.3

The data in table 1 show that there was no very significant difference in the thickness of the phloem cylinder in twigs from unsprayed and sprayed trees. The phloem of the older twigs was thicker than that of the younger ones. However, the xylem cylinder was distinctly thicker in the twigs from sprayed than from unsprayed trees. The averages for the young twigs showed no significant difference, but in the case of the two lots of older twigs the differences are statistically significant.

The 26-month-old twigs were nearly a year old before zinc sulphate was applied to the trees on which they grew. Hence, it is not surprising that the difference in size between sprayed and unsprayed twigs in this lot was somewhat less than in the 14-month-old lot.

These measurements may be interpreted to mean that the improvement in general growth activities already described extended to the activity of the cambium and resulted in the production of more wood in these twigs. Although too few data are available to permit a discussion of the specific action of the zinc on the cambium cells, it may be pointed out that the increased cambial activity would logically follow the great increase in chlorophyll-bearing area previously described and the general improvement in the condition of the trees elsewhere reported.[5]

SUMMARY

Orange trees affected with mottle-leaf produced healthy foliage and increased growth after they had been sprayed with a zinc-lime mixture. The specific effects observed were: (1) Larger leaves, (2) longer internodes, and (3) more xylem tissue.

[5] PARKER, E. R. See footnote 4.

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